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Effect Of Anaerobic Dairy Manure Co-Digestion And Effluent Solid Separation On Volatile Fatty Acids During Manure Storage

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EFFECT OF ANAEROBIC DAIRY MANURE CO-DIGESTION AND EFFLUENT SOLID SEPARATION ON VOLATILE FATTY ACIDS DURING MANURE STORAGE

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For my family and Jim and Deloris Holiman.

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ABSTRACT

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Volatile fatty acids (VFA) are among the most abundant organic compounds found in animal manure and act as important intermediates in the production of methane under anaerobic digestion (AD). However, VFA also contribute to odor emissions from manure. Anaerobic digestion and separation of solids may help to reduce odor pollution during manure storage and subsequent land application by reducing VFA concentrations.

Little information about the characteristics and concentrations of VFA in dairy manure related to AD is available. This thesis presents the results of VFA production during two three-month storage studies of dairy manure collected from four different sources on a dairy: a dairy barn (raw manure), the inlet of an anaerobic digester (AD influent), the digester outlet (AD effluent), and the effluent following solids separation (SS effluent). Significant temporal and spatial variations in VFA concentrations were observed in both studies. Results showed AD significantly reduced the formation of VFA in the effluent, and additional reductions occurred from separation of solids. This study revealed that the complexity of VFA characteristics made it difficult to reliably model and predict the concentrations and compositions of VFA in dairy manure.

CHAPTER 1. INTRODUCTION

Manure produced at animal facilities may be stored using a number of methods including basins, deep pits, and lagoons, and may be open or uncovered. Storage times vary, but during that time, manure undergoes natural degradation due to the presence of microorganisms. Manure contains undigested material including proteins, carbohydrates, and lipids that are degraded by microbial activity producing a variety of compounds that can negatively impact air quality including volatile organic compounds (VOC) (Peu et al., 2004). Volatile organic compounds can be divided into several subgroups including sulfurous compounds, phenols and indoles, and volatile fatty acids (VFA) (Le et al., 2005). Certain VOC are subject to government regulation, but analytical difficulties exist in quantification of VOC emissions (Alanis et al., 2010). Of the 500 VOC that have been identified at swine facilities, only a small portion of these compounds have been quantified (Ni et al., 2012). Quantification of VOC emissions is important for governmental regulation purposes, so many studies have focused on understanding which of the VOC are the most abundant. Several research groups have identified VFA as the most abundant VOC occurring in swine manure and at cattle feedlots (Ni et al., 2012; Trabue et al., 2011). VFA have also been found to be a useful indicator of total VOC content because VFA are produced through processes that lead to the formation of other VOC (Rabaud et al., 2003; Conn et al., 2007).

Additionally, volatile organic compounds have been closely correlated to odor at these facilities (Alanis et al., 2010). O'Neill and Phillips (1992) identified more than 100 odorous VOC from pig manure. Although odor is a complex mixture of

chemicals, four main groups of odor have been identified: sulfurous compounds, phenols and indoles, volatile fatty acids, and ammonia and volatile amines (Le et al., 2005). Currently, there are no federal and Indiana State regulations to control odor, yet a significant proportion of air pollution complaints are from nuisance odor, especially in areas around swine and dairy facilities (Sucker et al., 2009; Trabue et al., 2011). Because it is not practical to monitor all individual chemicals present in odors, research has been conducted to determine major indicators of malodors. Studies have found that ammonia and hydrogen sulfide were not suitable indicators for odor because the formation of either compound does not reflect the kinetics of manure degradation (Zhu et al., 1999; Spoelstra 1980). Sulfurous, phenol and indole compounds appear to contribute the most to malodor (Le et al., 2005), but some studies have demonstrated that VFA are strongly correlated with odor generation and can serve as a suitable odor indicator for manure stored both aerobically and anaerobically (Hobbs et al., 1998; Ndegwa 2003). Recent studies have shown that individual VFAs contribute differently to odor generation. Short chain fatty acids including acetic and formic acids may be present in manure in much higher concentrations, but long-chain and branching VFA have been shown to have more offensive odor (Hansen et al., 2006; Zhu 1999), making analysis of individual VFA necessary to assess potential for odor generation. However, due to VFA's affinity to absorb to surfaces, it can be difficult to make gas-phase measurements. Therefore, some studies of odorous compound emissions depended on VFA concentration in the liquid phase (Blanes-Vidal et al., 2009a). Recently, the use of VFA to monitor odor intensity and the effectiveness of techniques for odor reduction has become accepted by many researchers (Zhang and Zhu 2003; Miller and Varel 2001).

To produce renewable energy and mitigate these emissions, anaerobic digestion (AD) has been adopted as a technology that can be used to treat manure as well as other types of waste biomass and therefore help reduce the environmental impact of swine and dairy facilities (Shin et al., 2011). Anaerobic digestion

provides many benefits including odor reduction through degradation of key odorants, reduction of pathogens for manure that will be land applied, reduction in greenhouse gas emissions, lower sludge volumes (relative to aerobic treatment), and generate renewable energy through the production of biogas in the form of methane (Bond et al., 2012). The AD process relies on the symbiotic microorganisms present in manure, occurring in three main phases: hydrolysis, acidogenesis, and methanogenesis. During the first phase, hydrolytic bacteria secrete enzymes that help to solubilize the initial complex polymers present in manure including carbohydrates, lipids and proteins. These hydrolyzed compounds are then fermented to VFA by acidogenic bacteria. Carbohydrates are transformed to straight-chain VFA, while proteins can be transformed to both straight-chain and branched-chain VFA (Le et al., 2005). Acetogenic bacteria then convert VFA to acetate, hydrogen and carbon dioxide which are utilized by methanogenic archae to produce methane and carbon dioxide (Liu et al., 2012). Anaerobic co-digestion, where manure is simultaneously digested with various substrates including food wastes, energy crops, and agricultural wastes can increase biogas production by creating more favorable conditions in the manure, by changing such properties as moisture, alkalinity, and carbon:nitrogen (C:N) ratios (Bond et al., 2012; Frear et al., 2011). As an important intermediate in the AD process, VFA concentrations depend on substrate availability, the anaerobic flora present and the pH of the manure (Le et al., 2005).

In addition to AD, some facilities use solids separation as a method to further reduce odor from manure. Most of the odor-generating organic substances are produced from manure solids (Ndegwa et al., 2002). The separation of manure manure into a solid fraction and a liquid fraction has been found to reduce the potential of odor generation in stored animal manure (Hansen et al., 2006) by removing coarse particles that normally degrade slowly and enhancing the oxygen transfer efficiency and therefore improving the stabilization of the liquid manure (Ndegwa 2004). In an experiment conducted by Ndegwa (2002) solid-

liquid separation was performed on stored pig manure. Results showed a reduction in the production of volatile fatty acids in the first 30 days of storage.

Farms that utilize an AD system can differ in the management approaches for manure storage and treatment. Digesters may be different in design or operating temperature based on the type of manure, amount of manure, types of substrates, and region. Some studies revealed that there may be differences in the efficiencies of AD systems at reducing odor potential based on such management practices and environmental conditions (seasonal variation) (Lovanh et al., 2009). For those facilities that use co-digestion, potential inhibitory effects on methanogenic growth exist given the types of substrates used and the loading rates utilized (Frear et al., 2011). If microorganisms are not able to process all the incoming substrate, there may be undigested material still present in effluent manure. While stored, manure naturally undergoes AD in the layers below the shallow surface zone that's exposed to air (Lovanh et al., 2009). This leaves potential for the incomplete fermentation of those undigested materials by bacteria which results in the production of odorous compounds (Miller and Varel 2001).

Dairy manure "remains the foremost primary substrate for co-digestion due to its beneficial properties of high water content, good buffering capacity, and the presence of almost all the essential nutrients and trace elements" (Frear et al., 2011). In 2012, there were an estimated 168 AD systems in the United States, and 153 of them were on dairy farms (USEPA 2012). To assess the environmental impact of AD on odor pollution and air quality, it is important to characterize VFA in stored dairy manure related to AD. VFA are still one of the least-well characterized groups of compounds present in dairy manure (Alanis et al., 2010). There are only a few published studies on VFA in stored dairy manure. Patni and Jui (1985), El-Mashad (2011), and Moller (2004) conducted studies monitoring VFA characteristics in stored dairy-cattle manure, but only used raw

manure from dairy-cattle for testing. Miller and Varel (2001) studied VFA in fresh and aged (dried) manure from cattle. Frear (2011) conducted a study at a dairy manure-based AD facility focusing on differently treated manure but only reported the average concentration of 3 VFA during the test. In addition, no research in the literature has compared and characterized the VFA regarding their temporal and spatial variations in stored non-AD treated and AD-treated dairy manure.

The objectives of this study were to: (1) assess the effects of anaerobic dairy manure co-digestion and post-AD solids-liquid separation on select VFA, i.e., formic, acetic, propionic, butyric, and 2-methylbutyric acids during two storage tests; (2) study the dynamics of the changes in these select VFA during storage; and (3) compare two different analysis methods for determining VFA concentrations: HPLC and GC.

CHAPTER 2. GENERAL MATERIAL AND METHODS

2.1 Two Studies

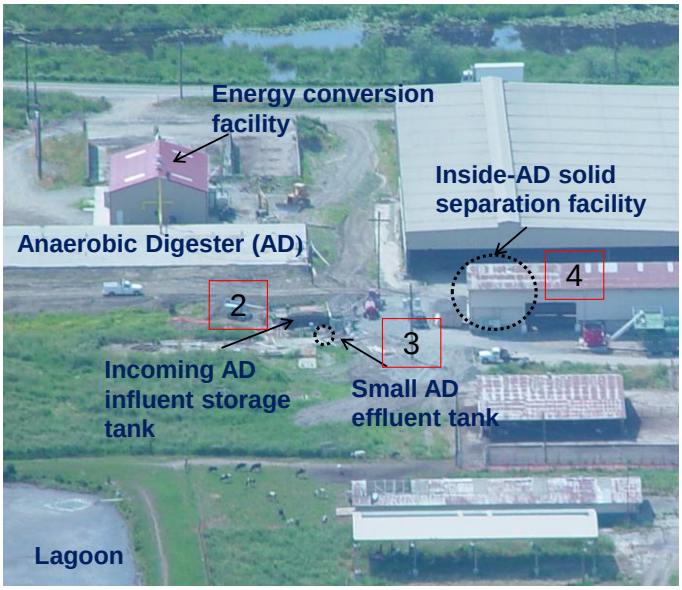
Two three-month storage studies of dairy manure were conducted. Manure was collected at two different times during the year, September 2011 (Test 1) and at the end of March 2012 (Test 2), and studied under the same lab conditions. Manure included a mixture of feces, urine, water and substrates in some cases.The only differences between the tests were the time of year manure was collected, the type of co-digestion material added to the manure at the time of collection, and the addition of continuous monitoring of the manure pH in the second test. The methods used for both tests are outlined below.

2.2 Digester Description

Dairy manure was collected from the Qualco anaerobic digester complex in NW Washington State. The digester receives manure from an 1100-head dairy that uses a flush manure management system. The manure was piped 1.6 km (1 mile) to the digester complex. The AD facility utilized a hybrid, plug-flow, complete-mix digester. The digester received “pre-consumer, organic waste-derived materials” (WSDA 2011) from surrounding businesses. The types of pre-consumer wastes that were added to the influent manure near the time of collection included blood from a ruminant slaughter plant, grease trap waste, waste (that was mostly glycerin) from biodiesel production, soiled bedding from the freestalls at the AD site, and fish processing waste.

2.3 Dairy Manure and Manure Preparation

For both storage tests, manure was collected from four different sampling locations, two undigested (untreated) and two digested (treated). The untreated sources included raw manure collected from a dairy barn and the influent (a mixture of dairy manure and pre-consumer waste) to the digester from the incoming storage tank (2, Figure 2.1). The treated sources included effluent from the AD system collected from the effluent tank (3, Figure 2.1) and liquid effluent from the AD system after solids-liquid separation (4, Figure 2.1).



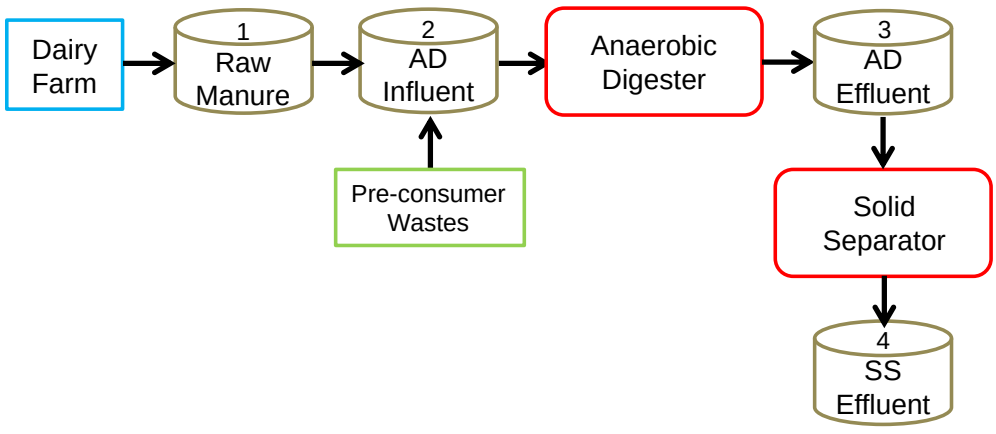


Figure 2.1. Top: Qualco anaerobic digester complex (photo from Washington State University). Bottom: Diagram of digester complex and sources.

Once collected, the four sources of manure were poured into storage containers that were sealed and frozen before shipping. When the containers were received at Purdue University, manure was allowed to thaw completely at room temperature before filling eight reactors (R1 to R8). Prior to filling, each container was mixed with a power mixer until the mixture was homogeneous. Manure was continuously stirred while loading the reactors to ensure uniformity in replicate reactors. The manure sources and reactor filling for both tests are listed in Table 2.1.

Table 2.1. Overview of manure preparation for both studies.

Container #	Sampling location	Reactor #	Reactor Filling	
			Test 1	Test 2
1	Raw manure from dairy barn	1 & 2	10/5/11 (d 0)	5/25/12 (d 0)
2	AD influent containing pre-consumer waste	3 & 4	10/5/11 (d 0)	5/25/12 (d 0)
3	AD Effluent	5 & 6	10/11/11 (d 6)	5/25/12 (d 0)
4	AD Effluent after separation of solids (input to a lagoon)	7 & 8	10/11/11 (d 6)	5/25/12 (d 0)

Each reactor was initially filled with manure to the maximum height allowed based on the volume of manure shipped and equal reactor volumes in all 8 reactors. The details of manure preparation and the laboratory setup can be found in section 3.2.2. In the second laboratory test, pH probes were installed in each reactor, allowing semi-continuous measurement of the pH in the top and bottom layers of manure.

2.4 Manure Sampling and Analysis

Two different types of analysis methods were performed on manure samples. The first type was a regular analysis of the manure that included total solids, total nitrogen, phosphate, sulfur, calcium, magnesium, sodium, iron, manganese, copper, zinc, pH and ammonium nitrogen. A regular analysis was conducted for samples collected from each container prior to filling to evaluate the initial

conditions of the manure and from each reactor at the termination of the experiment. Before taking samples for regular analysis, manure was completely mixed with a power mixer. In the second storage test, samples were taken 37 days after the end of the test for regular analysis. Regular analysis of all manure samples was conducted by an external laboratory (Midwest Laboratories, Inc., Omaha, NE).

The second analysis was conducted to determine VFA concentration. Manure samples were taken weekly from each reactor for VFA concentration analysis. Details of manure sampling for VFA analysis is described in section0. A breakdown of the number of samples and days taken is listed in Table 2.2.

Table 2.2. Overview of manure sampling schedule for both studies.

Test day		Regular samples		VFA samples		Operation
Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	
0	0	4	8	0	8	Test 1: Regular sampling of containers 1 and 2 (n=2) Test 2: Regular and VFA sampling of containers 1-4 (n=2).
6		4				Test 1: Regular sampling of containers 3 and 4 (n=2)
7 to 98	5 to 110			224	272	Both tests: Weekly VFA sampling of reactors (n=2)
107	131	8			16	Test 1: Regular sampling at end of test of 8 reactors (n=1) Test 2: VFA sampling at the end of entire test (n=2)

Figure 2.2. Manure sampling. Left: Sampling manure from the reactors. Right: Manure samples after collection from 12/21/11. The 8 samples in the top and bottom rows were taken from the top and bottom layer of the 8 reactors, respectively.

2.5 Analyses of Samples for VFA

High Performance Liquid Chromatography (HPLC) with a refractive index was used to determine the concentrations of five VFA in both studies (Figure 2.5). These VFA included formic, acetic, propionic, butyric, and 2-methylbutyric acids. Isobutyric acid was also included in the analysis, but because of an unknown interference, its concentrations could not be determined (Figure 2.3). More details of the HPLC method and equipment are presented in section 3.2.5.

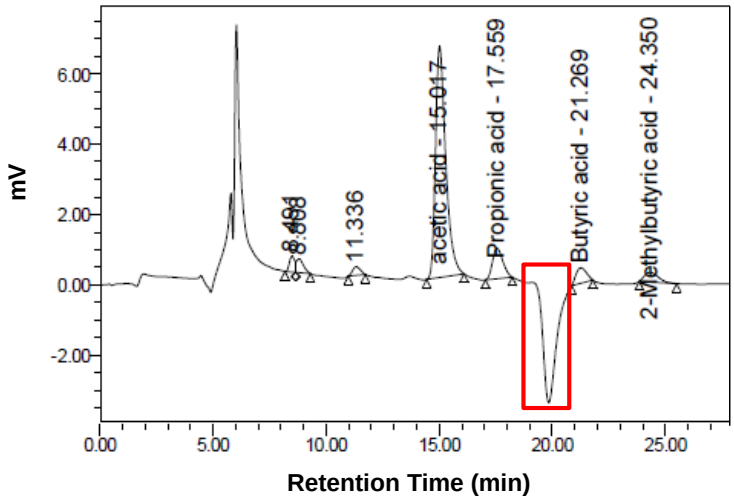


Figure 2.3. HPLC report showing interference at isobutyric acid retention time.

Sample preparation for the HPLC consisted of centrifugation and filtering. The manure samples were collected in 15 mL vials each week. These vials were centrifuged in a large centrifuge initially (Figure 2.4). Once centrifuged, each sample was then put into four 1.5-mL microfuges (Figure 2.4), which were then centrifuged in a smaller centrifuge. The liquid sample in the 4 microfuges was then transferred to 3 microfuges, which were again centrifuged in the smaller centrifuge. The samples were then filtered into the appropriate HPLC vials.

In the second study, 6 samples were analyzed using Gas Chromatography (GC) with a flame ionization detector in addition to the HPLC to compare the two methods (Figure 2.5). Details of the GC method and equipment can be found in section **Error! Reference source not found.**. Samples for GC analysis were repared the same way as for HPLC analysis, but an internal standard was added to each sample. The GC was able to determine the concentrations of isobutyric acid in the samples, allowing for the quantification of six VFA.

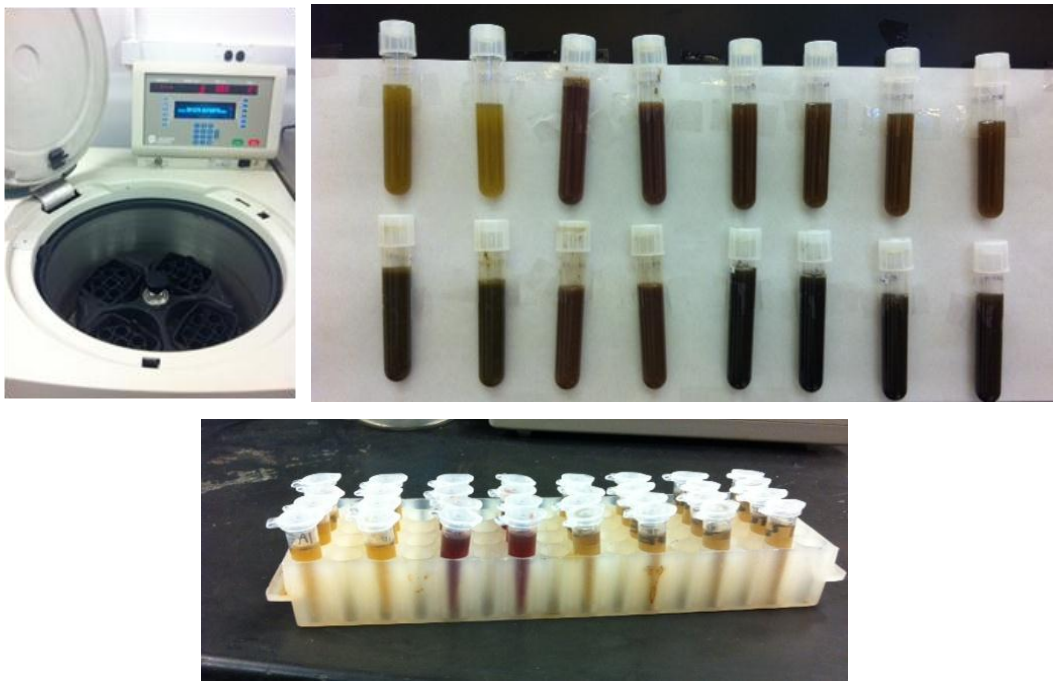


Figure 2.4. Manure preparation. Top left: Large centrifuge. Top right: Manure samples before centrifugation. Bottom: Manure samples in microfuges.

Figure 2.5. Equipment for VFA analysis. Left: HPLC. Right: GC.

CHAPTER 3. 2011 TEST[(1)]

3.1 Introduction

Manure naturally contains microorganisms that aid in manure degradation, but the breakdown results in the release of many compounds, including volatile organic compounds (VOC), than can negatively impact the environment. Volatile organic compounds are regulated by the U.S. EPA (CMA 1998) and can be divided into several subgroups, which include volatile fatty acids (VFA) (Schiffman et al., 1995). About 300 VOC have been detected and quantified at animal facilities and 36 VOC, many of them VFA, were detected in swine manure (Ni et al., 2012). Because VFA are usually among the most abundant VOC found in swine manure, they have been used as an indicator of the total VOC content in manure (Conn et al., 2007; Zhu et al., 1999).

Anaerobic digestion (AD) of dairy manure is an effective technology for generating renewable energy through the production of biogas that consists of 55-65% methane, as well as an important technology for the reduction of environmental pollution. Under anaerobic conditions, microbial decomposition of animal manure results in the production of VFA and other organic compounds (Cooper and Cornforth 1978). The VFA act as important intermediates in the production of methane. Acetogens further degrade VFA to acetate, carbon dioxide, and hydrogen. It is these products that are converted by methanogens to

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(1) This Chapter is a manuscript: Page, L., J.-Q. Ni, A.J. Heber, N.S. Mosier, X. Liu, H.-S. Joo, P.M. Ndegwa, and J.H. Harrison. “Volatile fatty acids in stored dairy manure before and after anaerobic digestion”, which has been submitted to *Journal of Environmental Management* and is currently under review. The preliminary version of this chapter was also presented in *ASABE Annual International Meeting*. Dallas, Texas, July 29 – August 1, as ASABE Paper No. 121337674.

methane (Gerardi 2003). It is recognized that methanogens can only use acetic acid, formic acid, and hydrogen directly, while butyric and propionic acids must first be converted to the former compounds by acetogenic bacteria (Dinopoulou et al., 1988). The microorganisms responsible for the production and consumption of VFA are sensitive to manure properties including pH, temperature, and ammonia nitrogen (Lu et al., 2008).

Biological degradation of manure can result in the release of odorous compounds (Mackie et al., 1998). Odors produced from manure are a complex mixture of ammonia, hydrogen sulfide, methane and VOC (El-Mashad et al., 2011; O'Neill et al., 1992). There are no federal guidelines to regulate and control odors in the environment. However, odors can be a nuisance and may create negative psychological responses by those impacted. Although it is the sulfurous, phenol, and indole compounds that appear to contribute the most to malodor (Le et al., 2005) some studies have demonstrated that VFA are strongly correlated with odor generation (Hobbs et al., 1998; Miller and Varel 2001; Ndegwa 2003). Le (2005), in a review article, concluded that there were significant differences between odorous compounds in general, and VFA in particular. The wide variations were most likely due to differences in sampling and measuring methods and different sources of samples. The use of multiple sources and replications of each source is beneficial to avoid wrong conclusions based on such differences. However, due to VFA's affinity to adsorb to surfaces, it can be difficult to make gas-phase measurements. Therefore, some studies of odorous compound emissions have been based on VFA concentration in the liquid phase (Blanes-Vidal et al., 2009b; Hansen et al., 2006).

Manure is usually stored on-farm under different management practices and environmental conditions before its use as fertilizer for crops. While stored, manure in the zone below the surface layer naturally undergoes AD (Lovanh et al., 2009; Cooper and Cornforth 1978). Characterizing VFA in stored dairy

manure related to AD is important for assessing the environmental impact of AD, including odor pollution and greenhouse gas emissions, because most of the successful agricultural AD systems in the U.S. are on dairy farms (USEPA 2010).

However, VFA are still one of the least well known groups of compounds present within dairy manure (Alanis et al., 2010; Rabaud et al., 2003; Sun et al., 2008). There are only a few published studies on VFA in dairy manure. In addition, no research in the literature has compared and characterized the VFA regarding their temporal and spatial variations in stored untreated and AD-treated dairy manure.

The objective of this chapter was to study the characteristics of five VFA, i.e., formic, acetic, propionic, butyric, and 2-methylbutyric acids related to the different manure sources from a digester complex and the treatments of these sources with AD and post-AD solids-liquid separation.

3.2 Materials and Methods

3.2.1 Dairy Manure and Manure Preparation

Dairy manure from four different sampling locations, two undigested (untreated) and two digested (treated), was collected from the Qualco anaerobic digester complex at the end of September 2011 in NW Washington, which was constructed in 2008 (WSDA 2011). The hydraulic retention time of the Qualco digester was approximately 16 days. The two untreated sources were the manure from a dairy barn (raw) and a mixture of dairy manure and “pre-consumer” wastes (WSDA 2011) to the digester (AD influent). The two treated manure sources were from the outlet of the digester (AD effluent) and the effluent after solids separation before storing in a lagoon (SS effluent).

The pre-consumer food processing wastes included several different biological wastes. Recorded daily inputs into the digester showed that, during the 16 days prior to the day of effluent manure collection for this study, the digester had been fed with 6.9% “Blood” that was waste from a ruminant slaughter plant; 1.2% “Fish” consisting of bread crumbs and fish waste from fish stick processing; 23.6% “Trap”, grease trap waste; and 68.3% dairy manure. On the day of the influent manure collection, the digester was fed with a mixture of 5.9% “Blood”, 4.0% “Trap”, and 90.1% dairy manure.

Samples of the four sources of manure were collected and frozen in four sealed plastic containers prior to shipment. The frozen containers were shipped to Purdue University where they were kept at room temperature for six days to thaw completely before filling eight manure-testing reactors (R1 to R8). Prior to filling, manure in each container was mixed with a power mixer until the mixture was homogenous. Manure was continuously stirred while loading the reactors to ensure uniformity in replicate reactors. Reactors R1 to R4 were filled on test day 0 and R5 to R8 were filled on day 6 (Table 3.1).

Table 3.1. Overview of manure preparation.

Container	Sampling date	Sampling location	Reactor	Reactor Filling
1	9/20/11	Raw manure from dairy barn	1 and 2	10/5/11 (d 0)
2	9/22/11	Influent to AD containing raw dairy manure and pre-consumer wastes	3 and 4	10/5/11 (d 0)
3	9/22/11	Effluent from AD	5 and 6	10/11/11 (d 6)
4	9/22/11	Effluent from AD after separation of solids	7 and 8	10/11/11 (d 6)

3.2.2 Laboratory Test of Simulated Manure Storage

Manure from each source was tested in two lab-scale reactors measuring 61 cm high and 38 cm diameter and made of white PVC. The inside surfaces of the reactors were lined with Tedlar films, except for the bottoms of the reactors. The

reactors were housed in a temperature-controlled walk-in chamber at about 20°C (Figure 3.1).

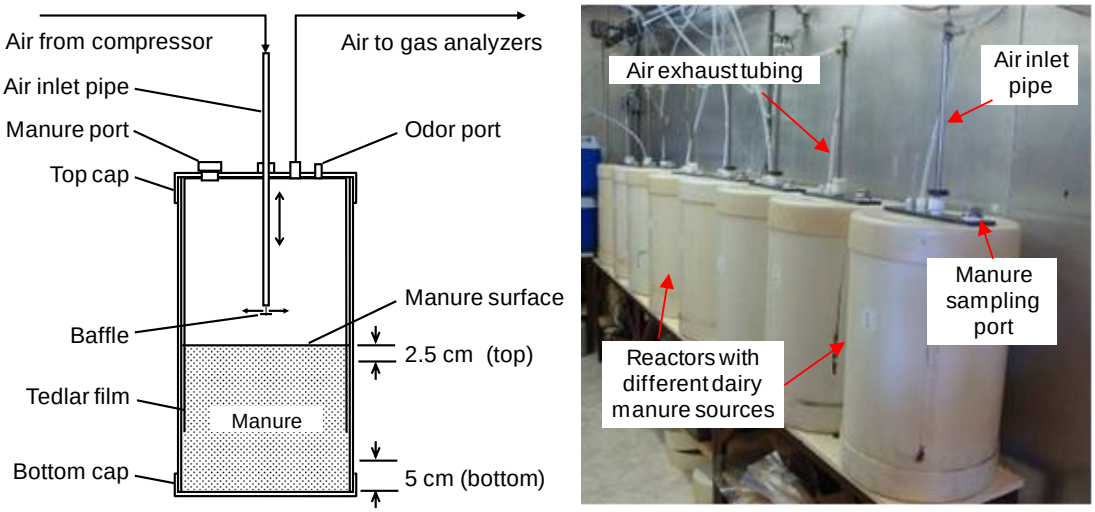

Figure 3.1. Reactor setup. Left: Cross section of reactor (not to scale). Right: Reactors filled with dairy manure under storage test in a temperature-controlled chamber.

Each reactor was initially filled with manure to a height of 25.4 cm and was continuously ventilated with 6.5 L min-1 of fresh air in the manure headspace for three months to simulate manure storage conditions on dairy farms (Figure 3.2). The exhaust air from each reactor and the reactor inlet fresh air were sampled weekly or biweekly for odor evaluation, and measured for 10 min approximately every 90 min for gas emission evaluation. Results of odor and gas emissions will be presented elsewhere.

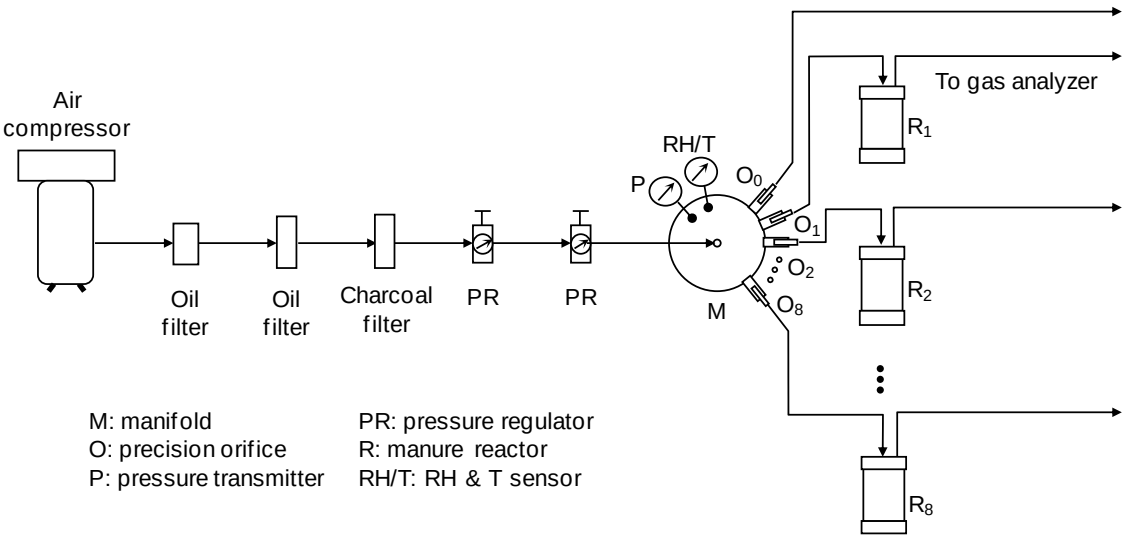

Figure 3.2. Schematic of the test setup (not to scale)

3.2.3 Manure Sampling for Regular Analysis

Before filling the reactors, two manure samples were taken from each container after the source manure was completely mixed with the power mixer on days 0 and 6. One manure sample was taken from each reactor at the end of the test on day 107 after the manure was completely mixed (Table 3.2). These 16 manure samples were shipped to an external laboratory (Midwest Laboratories, Inc., Omaha, NE) for analysis with a "basic manure package," which included moisture/total solids, total nitrogen, phosphate, potash, sulfur, calcium, magnesium, sodium, iron, manganese, copper, zinc, pH, and ammonia nitrogen.

Table 3.2. Overview of manure sampling schedule and total number of samples.

Test day	Regular samples	VFA samples	Operation
0	4		Regular sampling of containers 1 and 2 (n=2)
6	4		Regular sampling of containers 3 and 4 (n=2)
7 to 98		224	Weekly VFA sampling of reactors (n=2)
107	8		Regular sampling at the end-test of reactors (n=1)

3.2.4 Manure Sampling for VFA Analysis

Two manure samples were taken weekly for VFA concentration analysis from each reactor. A total of 224 samples were taken during the 3-month study (Table 3.2). One weekly sample was taken from the top manure layer within 2.5 cm below the surface and another was taken from the bottom manure layer within 5.0 cm above the reactor bottom (Figure 3.1). Samples were taken using 5-mL plastic pipettes. The manure height in each reactor was measured with a ruler prior to manure sampling. Based on the measurement, a pipette was marked accordingly to ensure the top layer was measured within 2.5 cm below the surface. Each sample contained 5 to 8 mL of manure depending on the amount of solids present in the sample. Samples with high solids content needed to be larger to ensure a sufficient amount of liquid portion for analysis.

3.2.5 Analyses of VFA Samples

Analyses of VFA were conducted via High Performance Liquid Chromatography (HPLC) using a Waters 2695 Separations Module (Waters Technologies Corporation, Milford, MA) with a Waters 2414 Refractive Index Detector at Purdue University's Laboratory of Renewable Resources Engineering. The column was a Bio-Rad Aminex® HPX-87H (Bio-Rad Laboratories, Hercules, CA), and the mobile phase was 5 mM aqueous sulfuric acid pumped at 0.6 mL min^{-1}. Manure samples were centrifuged for 10 min at 3,000 rcf (relative centrifugal force) followed by two 5-min successive sessions at 16,000 rcf to remove manure solids. Samples were then filtered using a 25-mm Nylon 0.2 µm filter before being analyzed with the HPLC system. Each samples was analyzed by injecting 50 µL of liquid. Data analyses of VFA were performed via Waters HPLC software (Empower, Waters Technologies, Milford, MA).

Concentrations of VFA were calculated after calibrations of all five acids were conducted using external standards (Figure 3.3). Linear regressions for each compound (determined by retention time) were obtained between peak areas for

three injections of samples containing known concentrations of each compound at four levels of concentrations. Mean differences among VFA concentrations within the same reactor and between replicate reactors were assessed with a general linear model (GLM) procedure using PROC GLM (SAS v.9.2, SAS Inst. Inc., Cary, NC).

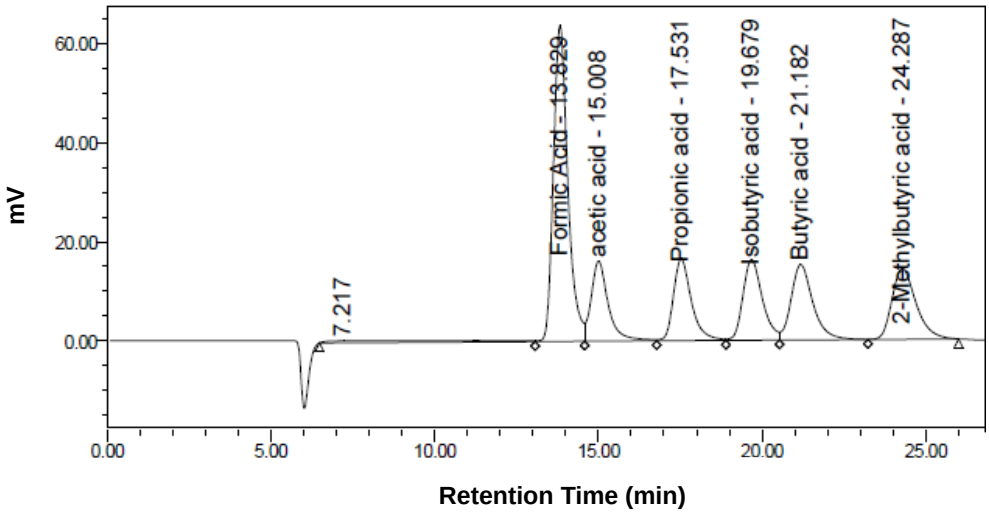

Figure 3.3. Acid standard test.

3.3 Results and Discussion

3.3.1 Characteristics of Source and End-Test Manure

3.3.1.1 Overview

The analysis of regular manure revealed differences among initial samples of all four types of source manure before the three-month laboratory test (Table 3.3). In the end-of-test manure samples, there were variations not only among the four groups, but also between the two replicate reactors within the same group. The properties of AD influent between the two reactors (R3 and R4) were the most different compared with the two replicates of the other three sources. The results demonstrated that the characteristics of manure could change substantially after months of storage and they could also be related to the characteristics of VFA in

the manure during storage.

Table 3.3. Results of selected parameters from regular analysis of the four manure sources.

Parameter	Raw Manure			AD Influent			AD Effluent			SS Effluent*		
	Initial	End		Initial	End		Initial	End		Initial	End	
	C1	R1	R2	C2	R3	R4	C3	R5	R6	C4	R7	R8
pH	8.3	8.4	8.4	4.4	6.2	5.5	8.3	8.5	8.6	8.4	8.6	8.7
Total Solids (TS), %	2.8	2.8	2.8	6.1	5.2	6.2	1.9	2.4	2.4	1.4	1.8	1.7
Ammonium Nitrogen (N), %	0.08	0.05	0.04	0.06	0.15	0.14	0.13	0.07	0.05	0.105	0.04	0.03
Organic Nitrogen (N), %	0.07	0.06	0.07	0.2	0.21	0.24	0.06	0.08	0.09	0.05	0.07	0.08
Total Kjeldahl nitrogen (TKN), %	0.15	0.11	0.11	0.26	0.36	0.38	0.19	0.15	0.14	0.16	0.11	0.11
Phosphorous (P_2O_5), %	0.08	0.10	0.10	0.10	0.11	0.14	0.07	0.08	0.08	0.05	0.07	0.06
Total Sulfur (S), %	0.01	0.02	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02

*Separation of Solids

3.3.1.2 pH

The pH of the influent ranged from 4.4 for the initial manure to 6.2 for the end-test manure in R3. This range of pH is close to the optimum pH for fermentative acidification necessary for the formation of VFA (pH of 5.7 to 5.9). The influent pH was much lower compared with the three other manure sources largely due to the pre-consumer wastes added into the influent. Studies have shown that accumulation of VFA result in a decrease in pH (Ghasimi et al., 2009; Ndegwa 2003) As pH decreases, the larger the proportion of VFA will be in the volatile nonionized form will increase (Conn et al., 2007).

The pH of raw and treated sources remained between 8.3 and 8.7. The lack of a significant change in pH may indicate a well-buffered manure solution (Patni and Jui 1985). However, by the end of the experiment there was an increase in pH of all sources. This may be due to the degradation of VFAs or protein which could have resulted in the production of ammonia to counterbalance VFA production (Yu and Fang 2001; Lu et al., 2008).

3.3.1.3 Total Solids

The analysis of total solids in the manure samples taken prior to reactor filling and at the end of the experiment showed a reduction in solids content in manure only for R3. The percent of total solids increased for treated sources and remained the same for raw manure. The increase in solids may be due to the loss of moisture through evaporation at the manure surface (Misselbrook et al., 2005). AD Influent had the highest initial total solids which could play a large role in microbial activities in the manure as well as the production of VFA (Zhu et al., 2001; El-Mashad et al., 2011) Emission of VOCs has been shown to be directly proportional to percentage solids in manure (Conn et al., 2007; Zahn et al., 2001).

3.3.1.4 Ammonium Nitrogen, Organic Nitrogen, and TKN

The highest TKN occurred in AD influent due to the high amount of organic nitrogen present. Organic nitrogen made up 60-70% of the TKN in AD influent and was three to four times higher than that of raw manure and the treated sources. Ammonium nitrogen (NH_4^+-N) decreased in all sources, except for AD influent, which more than doubled by the end of the test. The NH_4^+-N content in the AD influent at the end of the experiment was two to three times higher than the NH_4^+-N in the other three sources. Complex organic nitrogen compounds are mineralized to NH_4^+-N when digestion occurs, which may explain the increase in ammonium nitrogen in AD influent by the end of the test as well as the high initial ammonium nitrogen content present in treated manure (65-68% of the TKN in these sources) (Moeller and Mueller 2012). The amount of VFA and ammonia (NH_3) that are volatilized from manure mainly depends on the manure pH and concentrations of VFA and NH_4^+-N in the manure (El-Mashad et al., 2011).

3.3.1.5 Phosphorus and Total Sulfur

Phosphorus (P_2O_5) and total sulfur (S) concentrations increased from the initial samples to the end samples in all sources except for sulfur in the effluent (R5

and R6), which showed no change. The reason for this increase may be due to the loss of water that can be seen from the increase in total solids by the end of the test. AD Influent contained the highest amounts of both phosphorus and sulfur. Although the absolute concentrations were still low at the end of the test for all manure sources, the relative rate of increase was substantial for some reactors. For example, the sulfur concentrations in raw manure (R1 and R2) doubled after three months of storage. Nutrients such as these are needed for normal growth of the bacteria involved in AD (Ghasimi et al., 2009).

3.3.2 Characteristics of Individual VFA Concentrations

3.3.2.1 Overview

The five different VFA shown in the standard graph (Figure 3.3), including formic, acetic, butyric, propionic, and 2-methylbutyric acids, were detected in all four manure sources. The highest concentrations of VFA were found in the untreated AD influent (R3 and R4, Table 3.4). Previous experiments have revealed acetic and propionic acids as the main fermentation products (60-70% and 10-20%, respectively) from dairy manure (Cooper and Cornforth 1978; Dinopoulou et al., 1988; El-Mashad et al., 2011). In this study, however, acetic acid was the predominant VFA only in three sources (the raw manure and the two treated sources), but was not in the AD influent (R3 and R4). Propionic acid was the second predominant acid only in the raw manure (R1 and R2, Table 3.4).

The concentrations of five VFA exhibited temporal variations over the 3-month experiment. This agreed with several previous studies, which also showed that the proportion of individual VFA in manure could change over time due to different rates of degradation or formation between VFA. Patni and Jui (1985) reported the changes in VFA concentrations in dairy manure manure with different solids contents during undisturbed storage in covered tanks. Their results also showed that the concentration of the predominant VFA constituent

(acetic acid) governed the trend for changes in the concentration of total VFA in stored manure.

Moreover, the concentrations of the five VFA demonstrated spatial variations. The GLM analysis revealed significant differences ($p < 0.05$) in acid concentrations among all treatments, between replicate reactors with the same source manure, as well as within the same reactor. Most concentration differences were found in comparisons between the top and bottom layers of manure, demonstrating spatial variations in VFA concentrations in the manure.

The concentrations of VFA in the top layer were generally lower than in the bottom layer. This was most likely due to the more rapid decomposition of VFA by the aerobic and facultatively anaerobic or methanogenic bacteria due to exposure to air (Cooper and Cornforth 1978; Zhang and Zhu 2003; Patni and Jui 1985).

The weekly sample analyses revealed that although formic acid in R3 and R4, and acetic acid in R1 and R2 exhibited a general trend of decreasing in concentrations over time, the two VFA did not show the same tendency in all eight reactors. In addition, the concentrations of the other three VFA (propionic acid, butyric acid, and 2-methylbutyric acid) exhibited more irregular temporal variations. These characteristics of the five VFA made it difficult to reliably model and predict their dynamics in dairy manure.

Table 3.4. Mean ± standard deviation and range (in parentheses) of VFA concentrations in each reactor from 14 top layer (T) and 14 bottom layer (B) weekly manure samples.

Reactor	Layer	VFA concentration (mg L^{-1})					
		Formic Acid	Acetic Acid	Propionic Acid	Butyric Acid	2-Methylbutyric	Sum of 5 VFA
1	T	20±75 (0-281)	1298±967 (103-2595)	424±232 (0-729)	130±142 (0-501)	82±48 (0-127)	1867±648 (103-3677)
1	B	107±180 (0-528)	1424±1346 (76-3383)	661±347 (18-1381)	129±145 (0-348)	88±60 (0-155)	2302±804 (94-5365)
2	T	101±122 (0-334)	1098±941 (66-2312)	285±307 (0-875)	79±85 (0-204)	74±49 (0-129)	1567±588 (66-3432)
2	B	135±245 (0-827)	1318±1228 (78-2976)	552±337 (0-1149)	122±127 (0-291)	90±67 (0-183)	2119±737 (78-4704)
1 and 2	T and B	91±169 (0-294)	1284±1109 (81-2764)	480±332 (5-1008)	115±125 (0-260)	84±55 (0-144)	1963±685 (85-4223)
3	T	5545±9248 (0-21994)	2300±534 (1416-3343)	1488±354 (1028-2040)	2284±1104 (1299-4925)	294±201 (0-642)	11412±4417 (4417-29032)
3	B	7516±11197 (0-25720)	2992±481 (2264-3881)	1813±178 (1458-2146)	3047±1110 (1623-4958)	424±274 (43-740)	15102±5446 (5446-33029)
4	T	5117±8775 (0-21948)	2969±933 (1322-4423)	2103±244 (1844-2491)	3727±1631 (1153-6158)	280±165 (58-507)	13532±4224 (4224-28501)
4	B	7386±11226 (0-27075)	3399±1010 (1975-4873)	2265±422 (1337-2900)	4106±1756 (1713-6390)	317±194 (49-573)	16673±5483 (5483-34514)
3 and 4	T and B	6391±9950 (0-23529)	2915±853 (1872-3701)	1917±427 (1519-2097)	3291±1558 (1601-5388)	329±214 (54-561)	14175±4825 (4825-30648)
5	T	108±166 (0-550)	89±78 (0-203)	18±64 (0-240)	6±10 (0-28)	13±20 (0-62)	225±95 (0-550)
5	B	90±242 (0-867)	282±196 (55-584)	42±58 (0-217)	14±17 (0-42)	30±26 (0-72)	439±170 (75-1232)
6	T	2±4 (0-12)	76±96 (0-334)	1±4 (0-13)	4±9 (0-26)	8±14 (0-35)	89±51 (0-408)
6	B	124±312 (0-905)	237±186 (0-543)	16±23 (0-64)	13±16 (0-35)	26±25 (0-61)	400±181 (0-964)
5 and 6	T and B	90±214 (0-420)	171±171 (45-379)	19±46 (0-84)	9±14 (0-28)	19±23 (0-47)	286±98 (98-607)
7	T	2±7 (0-27)	42±92 (0-331)	1±4 (0-14)	4±9 (0-23)	6±13 (0-32)	55±43 (0-400)
7	B	99±251 (0-693)	175±231 (0-660)	6±13 (0-46)	7±15 (0-46)	15±17 (0-52)	293±163 (0-772)
8	T	0±0 (0-0)	61±111 (0-387)	2±8 (0-31)	6±10 (0-24)	8±13 (0-34)	76±54 (0-476)
8	B	0±0 (0-0)	225±238 (0-597)	12±23 (0-70)	14±18 (0-51)	16±21 (0-56)	257±135 (0-760)
7 and 8	T and B	25±130 (0-173)	126±192 (0-325)	5±14 (0-23)	8±14 (0-30)	11±16 (0-38)	169±80 (0-398)

3.3.2.2 Formic Acid

Formic acid is the simplest carboxylic acid. Reactors containing AD influent had extremely high concentrations of formic acid for the first four weeks. Its highest concentration was 27,100 mg L^{-1} at the bottom of R4 on day 14. The formic acid concentrations in R3 and R4 decreased to zero with very small fluctuations around day 56. Formic acid concentrations ranged from 0 to 905 mg L^{-1} in raw manure reactors and treated manure reactors (Figure 3.4). The most reasonable explanation for this is that the pre-consumer wastes added to the influent introduced substantial formic acid that was then broken down rapidly by microbial activity. The considerable reduction in formic acid concentrations in R3 and R4 after day 42 could be due to the initiation of methanogensis because methanogens can directly use formic acid (Dinopoulou et al., 1988).

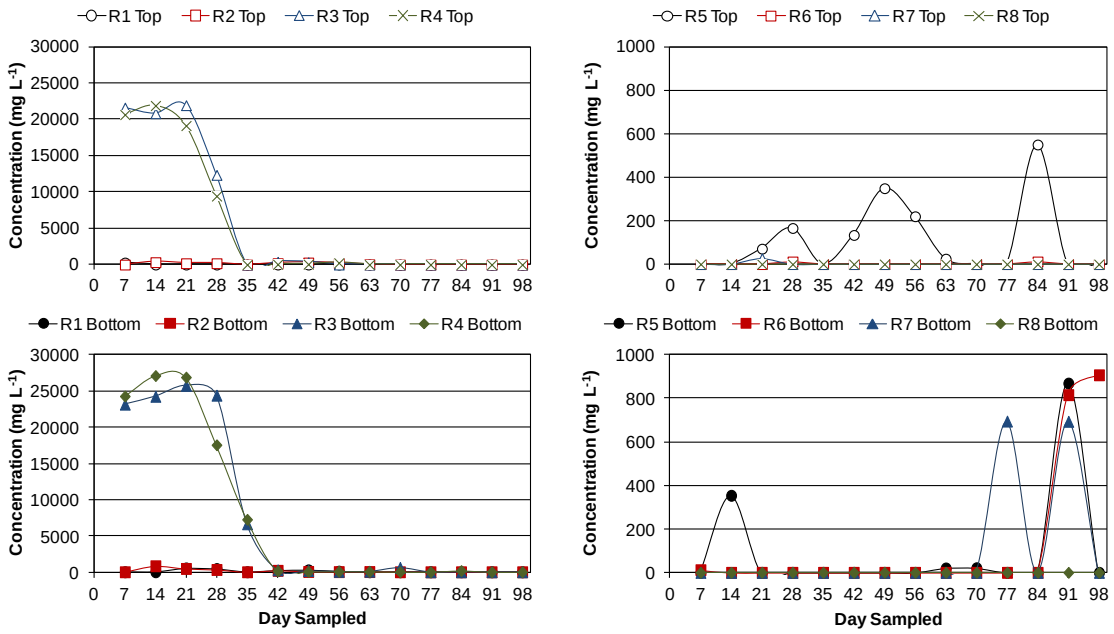

Figure 3.4. Comparison of formic acid concentrations in the four sources. Top Left: Top layer of untreated. Top Right: Top layer of treated. Bottom Left: Bottom layer of untreated. Bottom Right: Bottom layer of treated.

3.3.2.3 Acetic Acid

As the predominant VFA in most of the manure sources, concentrations of acetic acid increased in reactors containing raw manure (R1 and R2) until around day 21 and then declined steadily. Although acetic acid was not the predominant VFA in the AD influent (R3 and R4), its absolute concentrations in the influent were the highest among the four sources (Table 3.4). Concentrations of acetic acid in R3 top and bottom reached the maximum concentrations of 3340 and 3880 mg L^{-1} on days 21 and 28, respectively (Figure 3.5). The concentrations declined until day 49 and then gradually increased with some fluctuation. In R4, acetic acid concentrations began to decrease on day 21, but began to increase on day 42 and continued to increase until day 91, when the concentration dropped more than 2000 mg L^{-1}. The maximum acetic acid concentration among all reactors was 4870 mg L^{-1} at the bottom of R4 on day 84. There were large differences in acetic acid concentrations for the influent compared with the treated sources. The maximum acetic acid concentration in treated manure was only 660 mg L^{-1} and it occurred on day 70 at the bottom of R7 (SS effluent). Concentrations of acetic acid in both R5 and R6 (AD effluent) bottom layer increased after day 7, but began gradually decreasing after days 14 and 21, respectively. The top layers of the treated reactors all had lower concentrations of acetic acid compared with the bottom layers. The temporal and spatial variations in acetic acid concentrations in different reactors demonstrated characteristics that were more complex than previously reported.

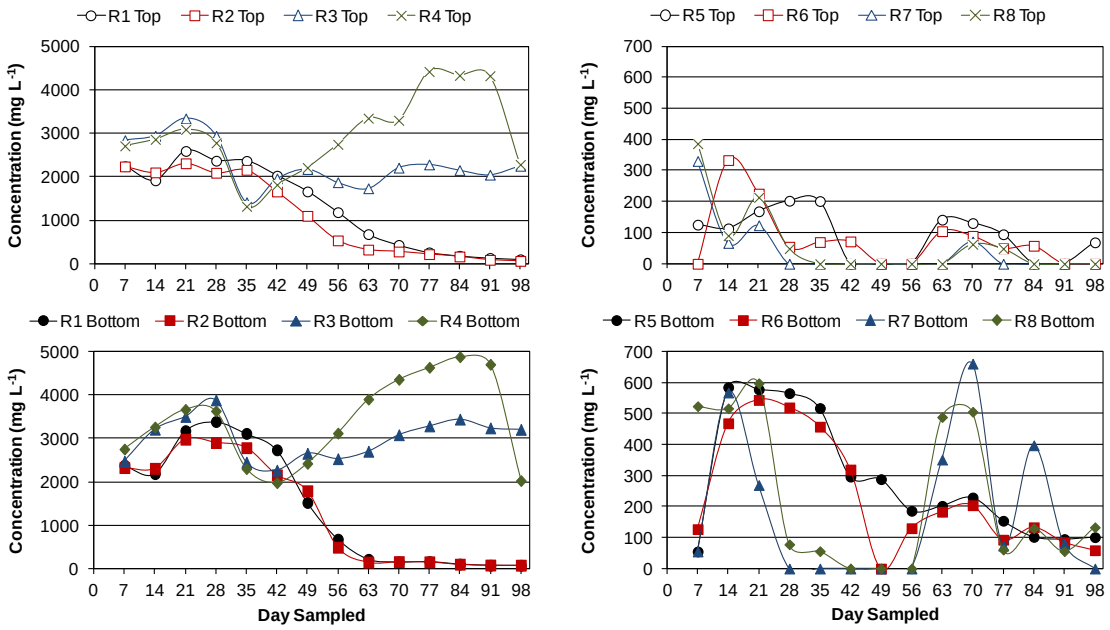

Figure 3.5. Comparison of acetic acid concentrations in the four sources. Top Left: Top layer of untreated. Top Right: Top layer of treated. Bottom Left: Bottom layer of untreated. Bottom Right: Bottom layer of treated.

3.3.2.4 Propionic Acid

Concentrations of propionic acid were highest in reactors containing AD influent (R3 and R4), reaching a maximum concentration of 2,900 mg L^{-1} on day 84 (Figure 3.6). The propionic acid concentrations in reactors containing raw manure (R1 and R2) increased until about day 35 and then completely degraded by the last day of sampling. Concentrations of propionic acid in treated manure reached a maximum of 240 mg L^{-1} in R5. Concentrations did not exceed 70 mg L^{-1} for R6, R7 and R8. Because R5 and R6 were replicates, the difference in propionic acid between the two reactors demonstrated a significant variation within the same treatment.

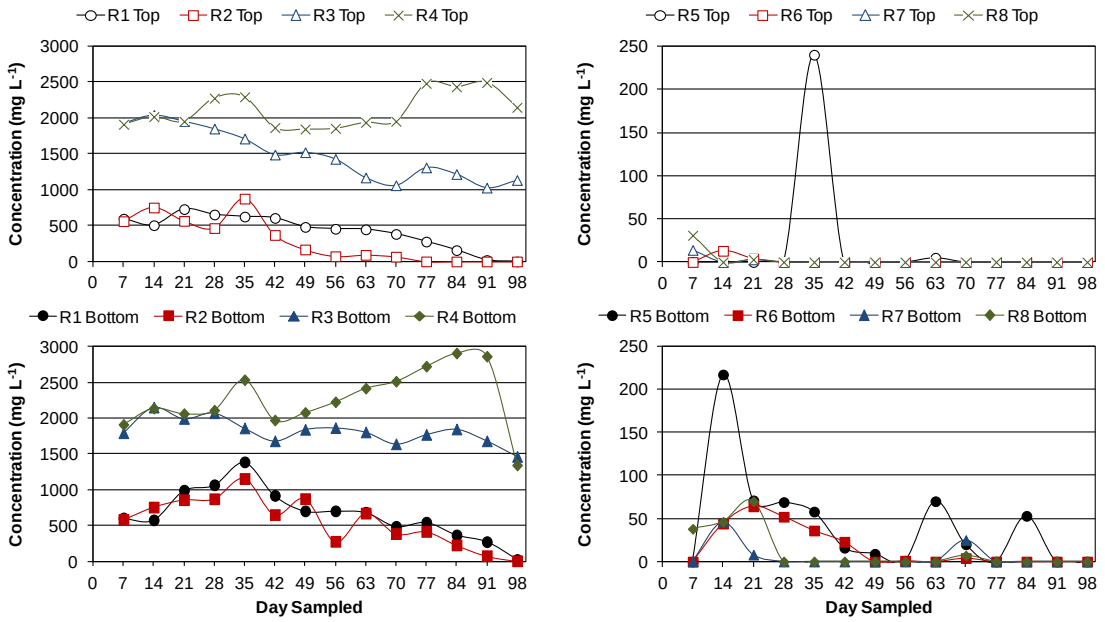

Figure 3.6. Comparison of propionic acid concentrations in the four sources. Top Left: Top layer of untreated. Top Right: Top layer of treated. Bottom Left: Bottom layer of untreated. Bottom Right: Bottom layer of treated.

3.3.2.5 Butyric Acid

Butyric acid concentrations ranged only from 0 to 501 mg L^{-1} for raw manure reactors (R1 and R2). Concentrations of butyric acid did not exceed 51 mg L^{-1} in any treated reactor (R5 to R8). Reactors containing the AD influent (R3 and R4) had the highest concentrations of butyric acid, ranging from 1,150 to 6,390 mg L^{-1}. In both the top and bottom layers of R3 and R4, butyric acid concentrations increased until days 35 and 42, respectively, then gradually declined (Figure 3.7). When comparing with the formic and butyric acids in R3 and R4, the concentrations of butyric acid did not begin to increase until formic acid concentrations declined. All correlation factors ranged from -0.969 to -0.985, showing high correlations between the two VFA, when the correlation coefficients for top and bottom layers in R3 and R4 were calculated for the period during which formic acid concentration reached 0 mg L^{-1} or when butyric acid reached its peak concentration. Formic acid accumulation can inhibit acidogenesis, and

those bacteria responsible for producing butyric acid seem to be more affected (Lu et al., 2008).

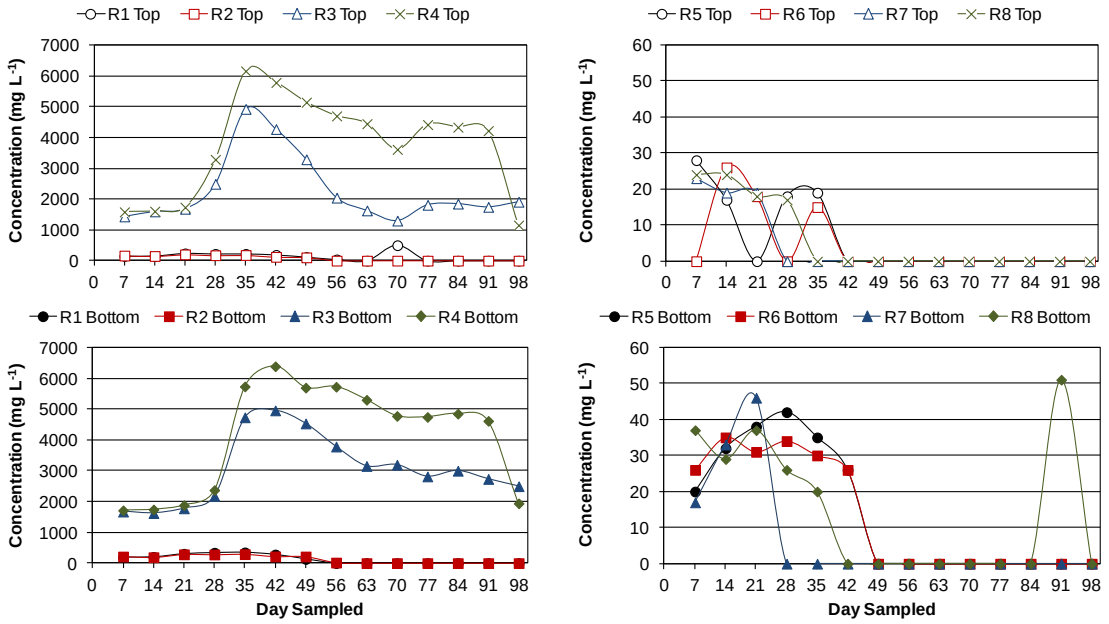

Figure 3.7. Comparison of butyric acid concentrations in the four sources. Top Left: Top layer of untreated. Top Right: Top layer of treated. Bottom Left: Bottom layer of untreated. Bottom Right: Bottom layer of treated.

3.3.2.6 2-methylbutyric Acid

The concentrations of 2-methylbutyric acid were very low compared with all other VFA. The maximum concentration of 740 mg L^{-1} occurred in R3 in the bottom layer on the last day of sampling. Concentrations of 2-methylbutyric acid in raw manure stayed fairly level, reaching a maximum concentration of 183 mg L^{-1}. On day 56, concentrations of 2-methylbutyric acid in raw manure began to decline until the VFA could no longer be detected. Concentrations in the AD influent were below those in raw manure until day 35 and continued to increase until day 84 for R4. At all time points and in both top and bottom layers, the concentrations of 2-methylbutyric acid in treated manure were below 75 mg L^{-1} (Figure 3.8).

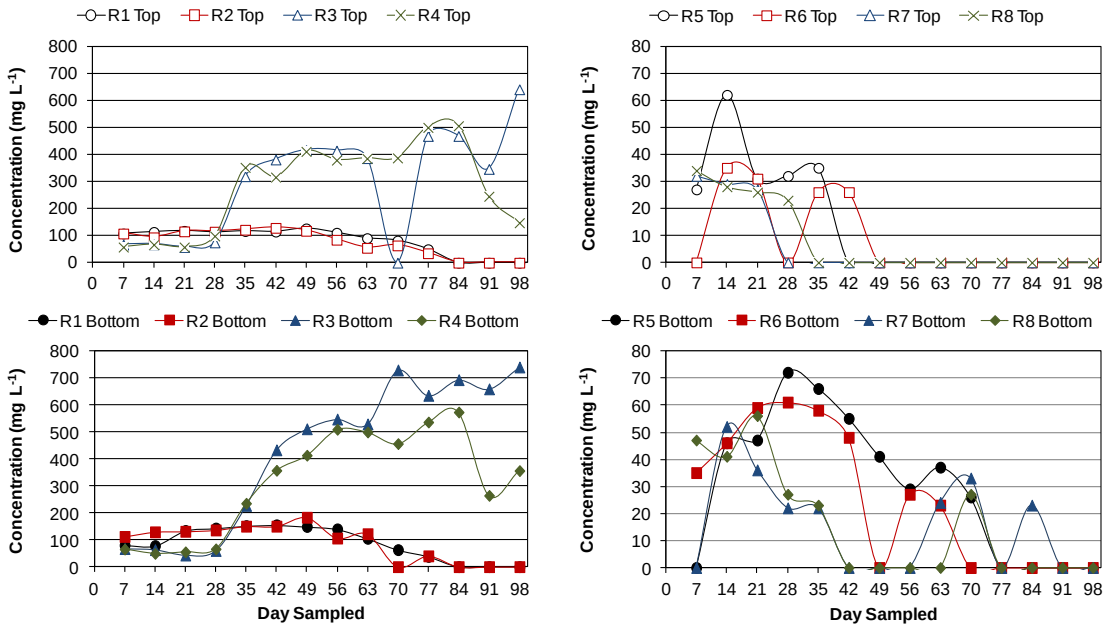

Figure 3.8. Comparison of 2-Methylbutyric acid concentrations in the four sources. Top Left: Top layer of untreated. Top Right: Top layer of treated. Bottom Left: Bottom layer of untreated. Bottom Right: Bottom layer of treated.

3.3.3 Effects of Manure Sources and Manure Treatment on VFA Concentrations

3.3.3.1 Effect of Pre-Consumer Wastes on VFA in AD Influent

The AD influent reactors (R3 and R4) had the highest VFA concentrations among all reactors (Table 3.4). Their total VFA of 14,175±4825 mg L^{-1} (mean±standard deviation) during the entire study was more than six times higher than that from the raw dairy manure (1963±4825 mg L^{-1}). In addition, while acetic acid was the most common VFA present in the other three sources, formic acid was dominant in the influent (R3 and R4). Furthermore, butyric acid concentrations were higher than acetic and propionic acid concentrations in the influent, instead of lower as in the other three sources. These characteristics showed that the addition of pre-consumer wastes in dairy manure in the influent resulted in not only higher VFA concentrations, but also different proportions of VFA compared with the raw dairy manure, which was dominated by acetic and propionic acids.

According to a recent study in Washington State (Frear et al., 2011), the addition of pre-consumer wastes can significantly increase biogas production over dairy manure alone. The main products of the first phase of AD, anaerobic acidogenesis, are acetic and butyric acids when the substrate added has easily degradable carbohydrates (Dinopoulou et al., 1988). The extremely high VFA concentrations in the influent in this study could provide some supporting evidence to the increased biogas production in Washington, where all of the operating dairy digesters use a combination of manure and some quantity of pre-consumer organic waste-derived materials (WSDA 2011).

3.3.3.2 Effect of AD and Separation of Solids on VFA in Stored Manure

The total VFA (sum of five VFA) presented in Table 3.4 and Figure 3.9 clearly show the differences among various manure sources. In general, the manure before AD (R1 to R4) had significantly higher total VFA compared with the manure after AD ($P<0.05$). Additionally, concentrations of all individual VFA in the treated manure never exceeded 1000 mg L^{-1} (Figure 3.4 to Figure 3.8) and were significantly lower than in untreated manure. This demonstrated that the Qualco AD system greatly reduced VFA concentrations in stored manure. Moreover, although both treated sources contained significantly lower concentrations of all five VFA compared with the untreated manure, separation of solids from the effluent further significantly reduced the total VFA concentrations (169±80 mg L^{-1}) compared with the effluent without solid separation (286±98 mg L^{-1}, $P<0.05$). However, due to the limitation of quantifying only five VFA in this study, future investigations are needed to determine the effect of AD and separation of effluent solid on other VFA that exist in dairy manure.

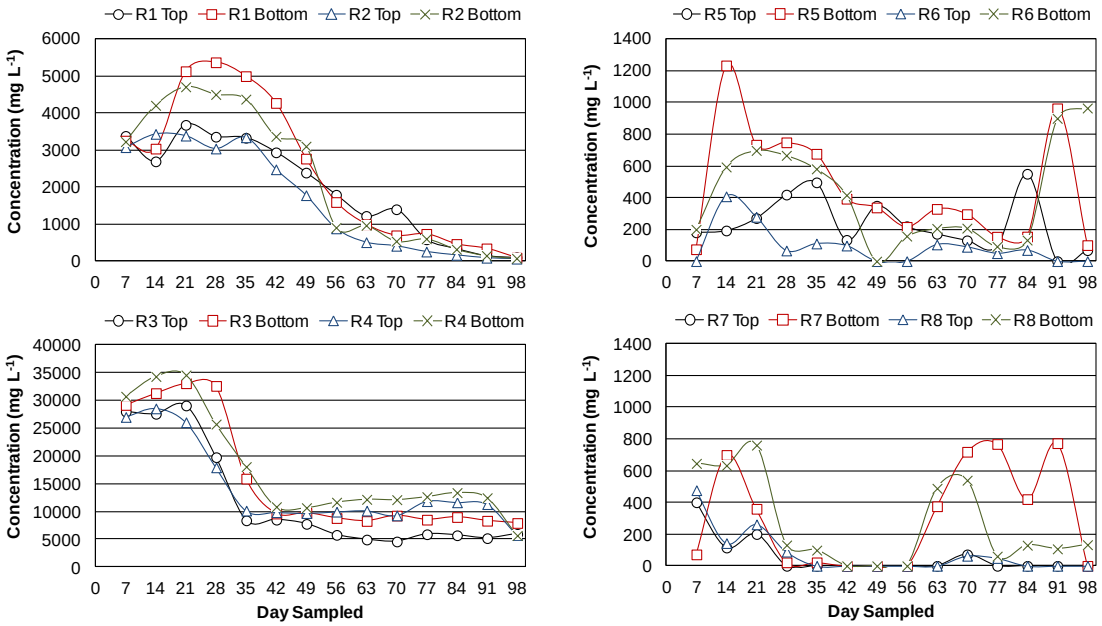

Figure 3.9 Variations of the total VFA concentrations in top and bottom layers in eight reactors. Top Left: Raw manure. Top Right: AD effluent. Bottom Left: AD influent. Bottom Right: SS effluent.

3.3.4 Comparisons with Reported Dairy Manure VFA

3.3.4.1 Comparison of VFA Concentration Variations

Limited data exists on VFA in dairy manure, and few present actual concentrations. El-Mashad (2011) collected raw dairy manure from a dairy barn and diluted it to 2%, 4%, and 9% total solids (TS) to test at temperatures of 15°, 25°, and 35°C under complete anaerobic conditions. The VFA measured by the El-Mashad (2011) included acetic, propionic, isobutyric, butyric, and valeric acids. The authors concluded that higher manure TS content and higher temperature resulted in higher VFA concentrations (Table 3.5).

Table 3.5. Comparison of VFA concentrations in dairy manure

Data source	TS, %[1]	Maximum concentration		Acetic acid / total VFA, %
		Total VFA	Acetic acid	
This study				
R1 top at 20° on day 21	2.8	3677 mg L^{-1}	2595 mg L^{-1}	71
R1 bottom at 20° on day 28	2.8	5365 mg L^{-1}	3383 mg L^{-1}	63
R2 top at 20° on day 14	2.8	3432 mg L^{-1}	Not maximum	N/A
R2 top at 20° on day 21	2.8	Not maximum	2312 mg L^{-1}	N/A
R2 bottom at 20° on day 21	2.8	4704 mg L^{-1}	2976 mg L^{-1}	63
El-Mashad et al. (2011) [2]				
At 25° on day 17	2	7930 mg COD L^{-1}	3569 mg COD L^{-1}	45
At 35° on day 6	2	7200 mg COD L^{-1}	3600 mg COD L^{-1}	50
At 15° on day 24	4	8600 mg COD L^{-1}	N/A	N/A
At 35° after day 30	4	16,500 mg COD L^{-1}	6930 mg COD L^{-1}	42
At 35° on day 10	9	23,000 mg COD L^{-1}	8000 mg COD L^{-1}	35

[1] TS in initial manure source. [2] Concentrations were not described numerically for all tested temperatures. Total VFA included acetic, propionic, iso butyric, butyric and valeric acids.

The maximum total VFA concentration was 7930 mg COD L^{-1} at 2% TS and 25 °C from the study of El-Mashad (2011). It was 48% and 59% higher compared with the raw manure in R1 (5365 mg L^{-1}) and R2 (4704 mg L^{-1}) of this study, respectively, at 2.8% initial TS and 20 °C in the bottom layer, where anaerobic conditions should persist. The differences were larger if it is compared with the top layers in R1 and R2. Moreover, the percentages of the maximum acetic acid in the maximum total VFA by El-Mashad (2011), which ranged from 35 to 50%, were lower than observed in this study, which ranged from 63 to 71%, at different test conditions (Table 3.5). These variations may be explained by differences in manure characteristics, individual VFA measured, and test conditions between the two studies, but also could have been due to some yet unknown factors that affected the microbial eco-systems in different manure reactors as demonstrated by the differences between paired reactors in this study. Nevertheless, the maximum concentrations of individual VFA in both studies occurred on different test days, showing the complex dynamics of VFA in dairy manure.

3.3.4.2 Comparison of Spatial and Temporal Variations in VFA Concentrations

In the study of Patni and Jui (1985), initial and final VFA (acetic, propionic, isobutyric, butyric, valeric, and iso-valeric acids) concentrations were determined in dairy manure in four 12.3 m x 7.2 m concrete tanks of 3.0 m depth at the

beginning and end of periods of 146 or 285 storage days. Manure samples were collected at regular intervals from each of the tanks at two locations and at depths of 0.3, 1.0, 1.8 and 2.5 m below the manure surface. The authors revealed that in all four tanks and for all VFA except iso-valeric acid, concentrations were significantly lower at the 0.3 m depth than at greater depths after about 50 days of storage. This top-low and bottom-high trend of spatial VFA concentration variation largely agreed with the results in this study as presented in the previous sections.

Additionally, Patni and Jui (1985) reported various patterns of individual VFA concentration changes over time in different tanks. Although the changes in VFA concentrations were very different in this study compared with those by Patni and Jui (1985) and the test conditions were very different (laboratory vs. field), both studies confirmed that there existed profound temporal VFA concentration variations in stored dairy manure.

3.4 Conclusions

1. Formic acid was dominant in the AD influent source. Its maximum concentration reached 27,000 mg L^{-1} in influent manure, compared with the maximum of <500 mg L^{-1} in the raw manure. Formic acid degraded rapidly in influent manure to about 200 mg L^{-1} in 6 weeks. However, its concentrations were more sporadic in treated manure.
2. Acetic acid was dominant in raw manure, AD effluent, and SS effluent. Acetic acid accounted for between 60% and 75% of the total VFA in these three sources, but was only 21% of the total VFA in the AD influent reactors. Except for the raw dairy manure where it demonstrated a general decrease during the study, the patterns of acetic acid concentration changes in other manure sources were irregular.
3. The maximum concentrations of propionic acid ranged from 730 to 2900 mg L^{-1} in untreated manure storage while never exceeding 240 mg L^{-1} in treated

manure storage. Propionic acid accounted for 24% of the total VFA in raw manure and weighed much less in the other sources.

4. Butyric acid was the second most dominant VFA and accounted for 23% of the total VFA in AD influent, but was only <6% in the other three sources. Concentrations of butyric acid and formic acid were highly correlated (correlation coefficients <-0.969) in the influent reactors, suggesting possible conversion of one to the other or concomitant competition.
5. Concentrations of 2-methylbutyric acid were the lowest among the five VFA in the untreated manure, but was similar to propionic and butyric acids in treated manure. Concentrations of 2-methylbutyric acid generally decreased in raw manure but were random in the other sources.
6. The pre-consumer wastes mixed with dairy manure not only increased the total VFA by more than 600% of the total VFA, compared with the raw dairy manure, but also changed the proportions of different VFA. Concentrations of formic and butyric acid were higher than the usual predominant VFA from dairy manure, acetic acid.
7. Concentrations of the total VFA in untreated treated manure exhibited a general decreasing trend over the three months of storage. However, changes in VFA concentration in the treated manure were more inconsistent and unpredictable.
8. Because VFA concentrations were significantly lower in the group of treated manure than in the group of untreated manure, this study demonstrated that AD significantly reduced VFA from dairy manure and pre-consumer wastes.
9. Most of the five VFA in the four different manure sources exhibited highly variable temporal and spatial differences. The characteristics of VFA revealed in this study were more complex than previously reported, lacking any real pattern and changing sporadically in some cases. This complexity makes it difficult to reliably model and predict the concentrations and compositions of VFA in dairy manure.

CHAPTER 4. 2012 TEST

4.1 Introduction

In the past decade, the number of successful dairy manure-based AD systems around the world has grown tremendously. The use of substrates for co-digestion in anaerobic digestion has helped to make biogas production from dairy manure more attractive by improving biogas yields. Although co-digestion is a favorable approach to improving biogas production and therefore making biogas plants more economically viable, certain challenges still exist. Selection of co-substrates, the amount of co-substrates added to manure, the organic loading rate, and the digester operation parameters affect the degradation mechanisms of the compounds present and ultimately affect methane production (Atandi and Rahman 2012). Degradation processes of certain substrates can result in the production of compounds that have inhibitory effects on methanogens. Many studies are being conducted to test different co-substrates under different operating conditions (Frear et al., 2011).

Although one of the benefits for AD is the potential for bioenergy, “odor concerns have been the main motivation for many of the existing digesters” (Lazarus 2008). As AD technology continues to change and develop, it will be important to continue evaluating the environmental impact of AD, including the effect on odor generation from stored manure, especially as affected by the addition of different substrates for co-digestion. As one of the compounds most closely correlated with odor from animal manure, VFA may serve as a suitable indicator to quantify odor. Quantification of VFA from manure before and after AD treatment will help to assess the digester function and evaluate the potential for odor generation.

Concentrations change during storage, and there's potential for VFA to accumulate in stored manure after treatment depending on digester efficiency.

There are several techniques for determining VFA concentrations in animal slurries. The most common and preferred method is gas chromatography (GC), but recently, HPLC methods have also been applied. The GC, with a packed or capillary column coupled with a flame ionization detector "allows a high resolution for fatty acid analysis in a complex mixture" (Peu et al., 2004). However, simple sample preparation coupled with a direct analysis when using HPLC also offers advantages. Preparing a sample for HPLC analysis may include only centrifugation and filtration. Determination using the GC method can also be simple, but the major disadvantage is the use of a derivative's agent in some cases (Siedlecka et al., 2008). In both methods, the amount of sample required is small. The GC can significantly lower the limit of quantification. In cases where concentrations are small this may be more beneficial. In a study performed by Siedlecka (2008) comparing three different methods: distillation, spectrophotometric, and GC methods, the GC method was determined to be the most reliable method for measuring low VFA concentrations.

The objective of this Chapter was to (1) repeat the experiment of characterizing the five VFA, i.e., formic, acetic, propionic, butyric, and 2-methylbutyric acids related to the different sources from a digester complex and the treatments of these sources with AD and post-AD solids-liquid separation, and (2) compare two analysis methods for VFA: HPLC and GC.

4.2 Materials and Methods

4.2.1 Dairy Manure and Manure Preparation

Dairy manure was collected from the same sources as the first storage test at the end of March 2012 in NW Washington State at the Qualco anaerobic digester

complex (See section 3.2.1 for details). Recorded daily inputs into the digester showed that, during the 16 days prior to the day of effluent manure collection for this study, the digester had been fed a mixture consisting of 2.7% “Blood” waste from a ruminant slaughter plant; 21.0% “Trap” that is grease trap waste; 2.7% “Biodiesel” that was a byproduct, which was largely glycerin, from crushing seed for biodiesel production; 8.8% “Bedding that is soiled bedding at the AD site that is dumped by a loader tractor into the receiving pit; and 67.4% dairy manure. On the day of the influent manure collection, the digester was fed with a mixture of 6.4% of “Blood”, 21.7% of “Trap”, 4.5% of “Bedding” and 64.8% of dairy manure.

The four sources of manure were poured into plastic containers that were sealed and frozen. The frozen containers were shipped to Indiana where they were kept at room temperature for one day and then put outside the second day to thaw completely before filling eight reactors (R1 to R8). Prior to filling, each container was mixed with a power mixer until the mixture was homogeneous. Manure was continuously stirred while loading the reactors to ensure uniformity in replicate reactors. The manure sources and reactor filling are listed in Table 4.1.

Table 4.1. Overview of manure preparation.

Container #	Sampling location	Reactor #	Received at PU	Reactor Filling
1	Raw manure from dairy barn	1 & 2	5/23/12 (d -2) and 5/24/12 (d -1)	5/25/12 (d 0)
2	Influent to AD containing raw dairy manure with food wastes and sludge	3 & 4	5/23/12 (d -2)	5/25/12 (d 0)
3	Effluent from AD	5 & 6	5/23/12 (d -2) and 5/24/12 (d -1)	5/25/12 (d 0)
4	Effluent from AD after separation of solids (output to a lagoon)	7 & 8	5/23/12 (d -2)	5/25/12 (d 0)

4.2.2 Laboratory Test of Simulated Manure Storage

See section 3.2.2 for details of the manure storage and lab setup. In this test each reactor was initially filled with manure to a height of 21.6 cm. The headspace of each reactor was continuously ventilated with 6.1 L min^{-1} fresh air from day 0 right after the reactors were all filled with manure. However, due to a mechanical malfunction, the air compressor was off from day 35 to day 52. During this period, the manure in the reactors was under completely anaerobic condition. Reactor headspace ventilation was restored on day 52 until day 114, when the air compressor was manually shut off until day 130 to re-create the 17-day manure anaerobic condition. The manure storage study was originally planned for 3 months. Because of the unexpected compressor failure and the subsequent compressor shut-off test, the entire study was extended to 130 days.

The exhaust air from each reactor and the inlet fresh air were sampled weekly or biweekly for odor evaluation, and measured for 10 min approximately every 90 min for gas emission evaluation, except during the compressor down-time. The study of odor and gas emissions is beyond the scope of this thesis.

Manure pH was measured using two pH probes that were installed in each reactor. One self-cleaning pH electrode (27003-12 Cole-Palmer, Vernon Hills, IL) was placed in the top 2.5 cm layer of manure. The height was adjusted when needed as the manure degraded and its volume decreased. A second submersible pH electrode (WD-35805-24 AKTON) was placed at 5 cm from the reactor bottom. The pH probe signals were acquired semi-continuously in both locations for 10 min each reactor, and approximately 16 times daily (Figure 4.1).

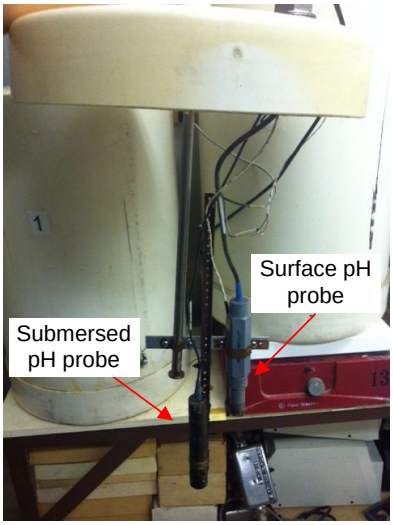

Figure 4.1. The pH probes attached to the reactor lid when they were pulled out of the reactor.

4.2.3 Manure Sampling for Regular Analysis

Before filling the reactors, two manure samples were taken from each container after the source manure was completely mixed with the power mixer (Table 4.2). These 8 manure samples were shipped to an external laboratory (Midwest Laboratories, Inc., Omaha, NE) for analysis of total solids, total nitrogen, phosphate, sulfur, calcium, magnesium, sodium, iron, manganese, copper, zinc, pH, and ammonium nitrogen.

Table 4.2. Overview of manure sampling schedule and total number of samples.

Test day	Regular sample	VFA samples	Operation
0	8	8	Regular and VFA sampling in containers 1-4 (n=2).
5 to 110		16/wk	Weekly reactor sampling (n=2)
131	16		Reactor sampling at the end of the test (n=2)

4.2.4 Manure Sampling and VFA Analysis

The procedures of manure sampling for VFA analysis were the same as in the first storage test and are described in Section 3.2.4. Analyses of VFA for all

samples were conducted via HPLC. See section 3.2.5 for details of the HPLC machine.

Six sample sets were also analyzed using an Agilent 7890 Gas Chromatograph equipped with a flame ionization detector, a model 7683B auto-sampler, and a 30 m x 0.25 mm x 0.25 µm Nukol column (Supelco, Bellefonte, PA). Helium was the carrier gas. The injector temperature was 250ºC, and the detector temperature was 300 ºC. The injection size used was 1 µL, using a split ratio of 30. Prior to GC analysis, samples were initially centrifuged for 10 min at 3,000 rcf (relative centrifugal force) followed by two 5-min successive sessions of 16,000 rcf. Samples were then filtered using a 25-mm nylon 0.2 µm filter. For each 1 mL of sample, 200 µL of an internal standard was added. The internal standard was a mixture of 100 mL of water and 0.2 g of 2-ethylbutyric acid.

Concentrations of VFA were calculated after calibration curves for all five acids were conducted using external standards. Linear regressions for each compound (determined by retention time) were determined between peak areas for three injections of samples containing known concentrations of each compound for three levels of concentrations. This method was used for both the GC and HPLC. For the HPLC results, peak integrations were in some cases corrected manually to ensure accurate peak detections.

Statistical analyses of the VFA concentrations from the HPLC results were performed using paired t-tests of the concentrations within the same reactor and between replicate reactors (MiniTAB v.16, Minitab Inc., State College, PA). Paired t-tests were also performed between the six sample sets that were run on both the GC and HPLC. For all the statistics, a significance level of $\alpha = 0.05$ was applied.

4.3 Results and Discussion

4.3.1 Characteristics of Source Manure

4.3.1.1 Overview

The analysis of regular manure revealed differences among initial conditions of all four types of source manure before the three-month test (Table 4.3). The properties of AD influent were the most different compared with the other three sources. This was most likely due to the addition of co-substrates in the influent.

Table 4.3. Results of selected parameters from regular analysis of the four manure sources.

Parameter	Raw manure	AD Influent	AD Effluent	SS Effluent
pH	7.9	7.3	7.9	8.0
Total Solids (TS), %	2.95	3.05	1.01	1.65
Ammonium Nitrogen (N), %	0.09	0.08	0.10	0.09
Organic Nitrogen (N), %	0.08	0.135	0.05	0.04
Total Kjeldahl Nitrogen (TKN), %	0.16	0.22	0.15	0.13
Phosphorous (P_2O_5), %	0.08	0.07	0.06	0.04
Total Sulfur (S), %	0.02	0.02	0.03	0.02

4.3.1.2 pH

The initial pH of AD influent was the lowest of the four sources, but this pH was much more basic compared to the initial pH for influent from the first storage test, suggesting a lower concentration of total VFA present in influent manure for the second storage test (this was later supported). Raw manure, AD effluent and SS effluent all had a similar initial pH, and the pH for all four sources was in the optimum range for methanogensis.

4.3.1.3 Total Solids

As expected the analysis of total solids (TS) in the samples prior to reactor filling showed a reduction in %TS after AD treatment. Raw manure contained about 3%

TS which is typical for a dairy farm that uses a flush system (El-Mashad et al., 2011). The %TS was not significantly higher in AD influent compared with raw manure, which may be explained by the large addition of grease trap waste as a substrate which has a high moisture content (Loustarinen et al., 2009). The results revealed that separation of solids did not have the expected effect on total solids, since the %TS was actually higher in effluent SS manure, which may be due to the method used when sampling at the digester site.

4.3.1.4 Ammonium Nitrogen, Organic Nitrogen, and TKN

The ammonium nitrogen (NH_4^+-N) content was very similar for all four sources, although slightly higher in treated manure. AD influent contained the highest TKN as well as organic nitrogen, most likely due to the addition of co-substrates. Overall, organic nitrogen content was higher in untreated sources compared with treated sources. Studies have found that organic nitrogen is mineralized to NH_4^+-N by microorganisms while in the digester (Moeller and Mueller 2012). This may explain the slight increase in NH_4^+-N as well as the lower organic nitrogen content in digested manure.

4.3.1.5 Phosphorus and Total Sulfur

The analysis showed that there was a slight reduction in phosphorous (P_2O_5) content after anaerobic digestion. The effect of AD on phosphorous availability has been subject of much debate, but in a review conducted by Moeller and Mueller (2012), it was learned that AD results in a small loss of phosphorous which supports the results of the analysis. The results in this research also show a small reduction in phosphorous after separation of solids. Previous studies have shown that the majority of phosphorous will remain in the solid fraction of manure after solid-liquid separation (Moeller and Mueller 2012). The amount of sulfur (S) observed in the four manure sources was very similar, although there was a slight increase in sulfur after AD and a small loss after separation of solids. Some loss can be expected from the digested manure due to a high proportion of potentially volatile S compounds (Moeller and Mueller 2012).

4.3.2 Overview of Manure pH

The results from the pH measurements taken within the reactor during the 3-month study exhibited both spatial and temporal differences between the top and bottom layer pH for all four manure sources (Table 4.4). However, certain similarities existed in the pH changes within manure of the same treatment groups (untreated and treated). The bottom layer pH remained lower than the top layer pH for all sources during the 3-month storage (Figure 4.2). These differences in pH are due to the differences in both the microbial population and also the chemical composition of the top layer exposed to air and the bottom anaerobic level (Lovanh et al., 2009). The pH of the bottom layer in untreated slurries remained between 6.5 and 8 which is the optimum pH for anaerobic digestion (Atandi and Rahman 2012). After the period of complete anaerobic conditions from days 35 to 52, when air flow was restored on day 52, there was an increase in pH for untreated manure, which was most likely due to the degradation of VFA that occurred (Yu and Fang 2001; Lu et al., 2008). During anaerobic conditions, reactors containing treated manure experienced a decline in pH, followed by an increase in pH once airflow was restored. This was not supported by changes in VFA concentration, so the decline in pH was most likely due to other pH controlling factors such as carbonic acid-bicarbonate buffers and ammonia (Conn et al., 2007; Patni and Jui 1985). Overall there were no significant changes in pH of any source. This may indicate that all manure sources were well buffered (Patni and Jui 1985). The data from days 82 and 89 was not used for the top layer of reactors 3, 5 and 7 because the probe was not touching the manure surface during this time.

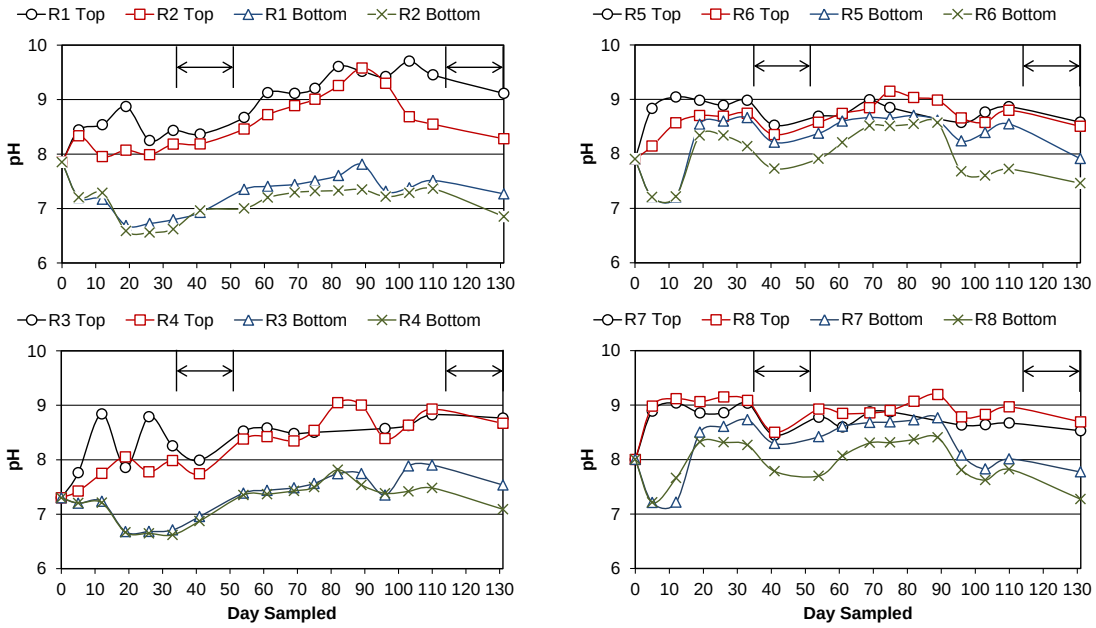

Figure 4.2. Variations in manure pH in the top and bottom layers in eight reactors. Top Left: Raw manure. Top Right: AD effluent Bottom Left: AD Influent. Bottom Right: SS effluent. The arrows indicate the time reactors were under anaerobic conditions.

Table 4.4. Mean ± standard deviation and range (in parentheses) of pH in each reactor from top layer (T) and bottom layer (B).

Location	pH			
Untreated				
Reactor	1	2	3	4
T	8.9±0.5 (7.9-9.7)	8.5±0.5 (7.9-9.6)	8.4±0.5 (7.3-8.8)	8.3±0.5 (7.3-9.0)
B	7.3±0.3 (6.7-7.9)	7.1±0.3 (6.6-7.9)	7.3±0.4 (6.7-7.9)	7.2±0.3 (6.6-7.8)
Treated				
Reactor	5	6	7	8
T	8.7±0.3 (7.9-9.0)	8.6±0.3 (7.9-9.1)	8.7±0.3 (8.0-9.0)	8.9±0.3 (8.9-9.2)
B	8.3±0.5 (7.2-8.7)	8.0±0.5 (7.2-8.6)	8.2±0.5 (7.2-8.8)	8.0±0.5 (7.2-8.4)

4.3.3 Characteristics of Individual VFA Concentrations

4.3.3.1 Overview of VFA

The four different VFA (Table 4.5), including acetic, propionic, butyric and 2-methylbutyric acids, were detected in all four sources. Formic acid was not present in any samples analyzed in the second storage test. The highest

concentrations of VFA were found in AD influent (R3 and R4, Table 4.5). Acetic acid was the predominant VFA in all sources, accounting for 66.2-82.9% of the four VFA during storage. Propionic was second most dominant VFA (6.9-19.6%) for raw manure, AD influent and SS effluent, while butyric was the second most dominant VFA for AD effluent accounting for 8.8-21.5%. Acetic and propionic acids have been reported as the main fermentation products from dairy manure in other studies (El-Mashad et al., 2011; Cooper and Cornforth 1978).

The concentrations of the four VFA exhibited temporal variations over the 3-month experiment. This agreed with the first storage test and previous studies which have also shown that the proportion of individual VFAs in manure can change temporally due to different rates of degradation or formation between VFAs (Moller et al., 2004; Conn et al., 2007).

Concentrations of VFA also exhibited spatial variations. The paired t-tests revealed significant differences ($p<0.05$) in acid concentrations among all treatments, between replicate reactors with the same source manure, as well as within the same reactor. Most concentration differences were found in comparisons that included the top layer of manure. The concentrations of VFA in the top layer were generally lower than in the bottom layer, similar to the first storage test, most likely due to more rapid decomposition of VFA because of air exposure (Patni and Jui 1985). The temporary anaerobic conditions from day 35 to 52 had a greater effect on the top layer of manure in some reactors, resulting in sudden changes in VFA concentration. However, certain reactors of the same source displayed the same concentration range in all layers. This occurred when the maximum concentration exhibited was the initial concentration in the container of source manure, before filling the reactors (Table 4.5).

Table 4.5. Mean ± standard deviation and range (in parentheses) of VFA concentrations in each reactor from 17 top layer (T) and 17 bottom layer (B) weekly manure samples.

Reactor	Layer	VFA concentration (mg L^{-1})				
		Acetic Acid	Propionic Acid	Butyric Acid	2-Methylbutyric	Sum of 4 VFA
1	T	422±410 (0-1519)	103±190 (0-603)	72±154 (0-640)	66±146 (0-637)	663±792 (0-2701)
1	B	714±845 (0-2388)	234±355 (0-1074)	106±184 (0-640)	75±147 (0-637)	1130±1395 (0-3763)
2	T	639±664 (0-1841)	195±244 (0-756)	88±160 (0-640)	75±147 (0-637)	997±1069 (0-2837)
2	B	853±951 (0-2620)	328±368 (0-1052)	115±177 (0-640)	87±147 (0-637)	1383±1498 (0-3884)
1 and 2	T and B	657±694 (0-1892)	215±272 (0-787)	95±164 (0-640)	76±146 (0-637)	1043±1140 (0-2894)
3	T	940±939 (0-2864)	328±477 (0-1464)	156±231 (0-664)	147±168 (0-473)	1571±1744 (0-5350)
3	B	1082±1131 (0-3178)	455±527 (0-1467)	198±276 (0-844)	163±175 (0-473)	1898±2028 (0-5958)
4	T	865±866 (0-2592)	303±413 (0-1417)	147±213 (0-659)	136±159 (0-473)	1451±1575 (0-5071)
4	B	1150±1173 (0-3163)	450±552 (0-1613)	212±284 (0-813)	167±176 (0-473)	1979±2113 (0-5971)
3 and 4	T and B	1009±1006 (0-2754)	384±482 (0-1485)	178±247 (0-695)	153±167 (0-473)	1724±1832 (0-5353)
5	T	122±162 (0-630)	34±101 (0-427)	34±106 (0-443)	31±100 (0-426)	222±444 (0-1926)
5	B	143±166 (0-630)	36±101 (0-427)	39±106 (0-443)	37±99 (0-426)	255±441 (0-1926)
6	T	140±161 (0-630)	34±101 (0-427)	30±105 (0-443)	36±99 (0-426)	240±443 (0-1926)
6	B	141±163 (0-630)	34±101 (0-427)	30±106 (0-443)	36±99 (0-426)	241±444 (0-1926)
5 and 6	T and B	137±161 (0-630)	34±101 (0-427)	33±105 (0-443)	35±99 (0-426)	239±442 (0-1926)
7	T	180±199 (0-617)	38±86 (0-357)	22±93 (0-395)	39±89 (0-384)	279±425 (0-1727)
7	B	173±189 (0-610)	36±85 (0-357)	22±93 (0-395)	44±95 (0-384)	275±426 (0-1727)
8	T	146±180 (0-619)	37±85 (0-357)	22±93 (0-395)	36±90 (0-384)	241±415 (0-1727)
8	B	138±147 (0-592)	35±83 (0-357)	22±93 (0-395)	34±89 (0-384)	229±395 (0-1727)
7 and 8	T and B	159±171 (0-592)	36±84 (0-357)	22±93 (0-395)	38±90 (0-384)	256±409 (0-1727)

4.3.3.2 Acetic Acid

The predominant VFA present in all sources was acetic acid (66.2-82.9%). The raw manure received from Washington State contained initial acetic acid concentrations of 821 mg L^{-1}. Once the raw manure was in the reactors, concentrations increased until day 19, and then began to decline. For raw manure reactors, the maximum concentration of 2620 mg L^{-1} occurred on day 19 in the bottom layer of Reactor 2. Concentrations in reactors containing AD influent followed a similar pattern as raw manure reactors; however the initial container concentration was 1120 mg L^{-1}. The maximum concentration, 3180 mg L^{-1}, in influent occurred on day 19 in the bottom layer of Reactor 3. For both untreated sources, a peak occurred in the top layers during the period of anaerobic conditions suggesting a shift in microbial activity that allowed for the accumulation of acetic acid (Patni and Jui 1985). Concentrations dropped to 0 mg L^{-1} by day 110 for both untreated sources. For reactors containing AD effluent and SS effluent slurries, the concentrations of acetic acid showed a general pattern of decline after the reactors were filled. The maximum concentration for AD effluent was the initial concentration of 630 mg L^{-1} in the container. Concentrations steadily declined until day 26 when a stable concentration between 100-200 mg L^{-1} was maintained for around 8 weeks. Concentrations reached 0 mg L^{-1} by day 89. For reactors containing SS effluent, the maximum concentration of 610 mg L^{-1} occurred on day 12 in the bottom layer of Reactor 7. Concentrations reached 0 by day 96 for both SS effluent reactors (Figure 4.3).

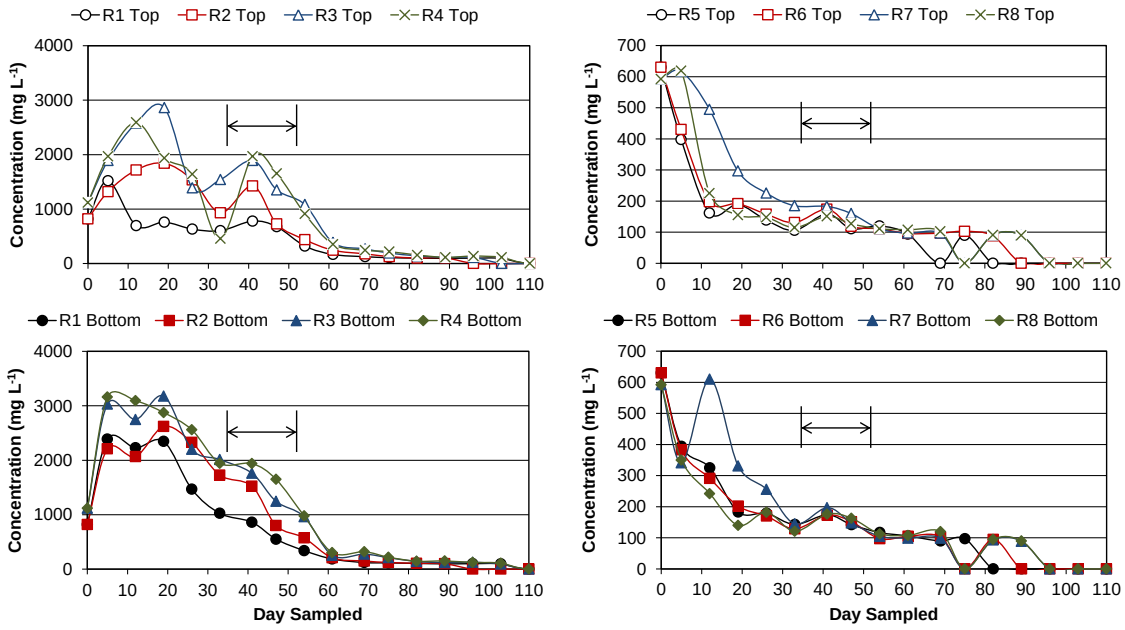

Figure 4.3. Comparison of acetic acid concentrations in the four sources. Top left: Top layer of untreated. Top Right: Top layer of treated. Bottom Left: Bottom layer of untreated. Bottom Right: Bottom layer of treated. Arrow indicates time of anaerobic conditions.

4.3.3.3 Propionic Acid

Propionic acid was the second most predominant VFA present in all sources (6.9-19.6%) except for effluent which had a higher percentage of butyric acid. Concentrations in reactors containing raw manure showed a pattern of decline after filling in the top layer, while concentrations in the bottom layer increased until day 12 and then declined. The maximum concentration occurred on day 12 in the bottom layer of Reactor 2, reaching 1070 mg L^{-1}. For AD influent, concentrations increased after filling until days 12 and 19. The maximum concentration, 1610 mg L^{-1}, occurred on day 12 in the bottom layer of Reactor 4. Both untreated sources showed a small peak in concentration of propionic acid during the anaerobic period in layers as well as a decline in concentration to 0 mg L^{-1} by days 75 and 82 of storage. For both treated sources, propionic acid concentrations declined sharply after 5 days and stayed very low until reaching 0

mg L^{-1} around day 41. The maximum concentrations were the initial container concentrations of 427 mg L^{-1} and 357 mg L^{-1} for effluent and SS effluent manure, respectively (Figure 4.4).

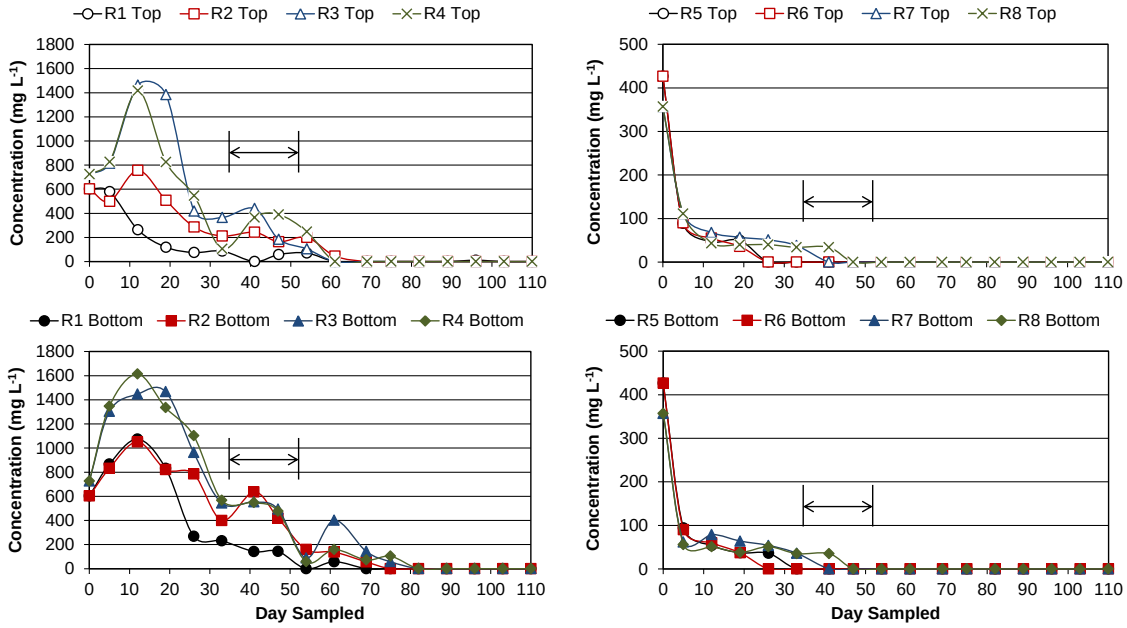

Figure 4.4. Comparison of propionic acid concentrations in the four sources. Top left: Top layer of untreated. Top Right: Top layer of treated. Bottom Left: Bottom layer of untreated. Bottom Right: Bottom layer of treated. Arrow indicates time of anaerobic conditions.

4.3.3.4 Butyric Acid

Reactors containing raw manure showed a pattern of decline in butyric acid concentration from the initial filling, declining rapidly within 5 days of storage. Concentrations declined to 0 mg L^{-1} by day 47 (during the anaerobic period). The maximum concentration of butyric acid in these reactors was the initial concentration in the container of 640 mg L^{-1}. The highest concentration of butyric acid (844 mg L^{-1}, Table 4.5) occurred in AD influent. For reactors containing influent, concentrations declined from the initial storage within 5 days, but then increased, reaching a maximum peak in concentration on days 19 and 12 in Reactors 3 and 4, respectively. The highest concentration occurred on day 19 in

the bottom of Reactor 3. Concentrations then declined sharply within one week and continued to decline reaching 0 mg L^{-1} by day 54. Both untreated sources experienced a small peak in concentration on day 41 during the anaerobic period. For both treated sources, butyric acid concentrations declined to 0 mg L^{-1} within 5 days of reactor storage. Random peaks occurred in effluent towards the end of storage (day 96).The maximum concentrations were the initial container concentrations of 443 mg L^{-1} and 395 mg L^{-1} for effluent and SS effluent, respectively (Figure 4.5).

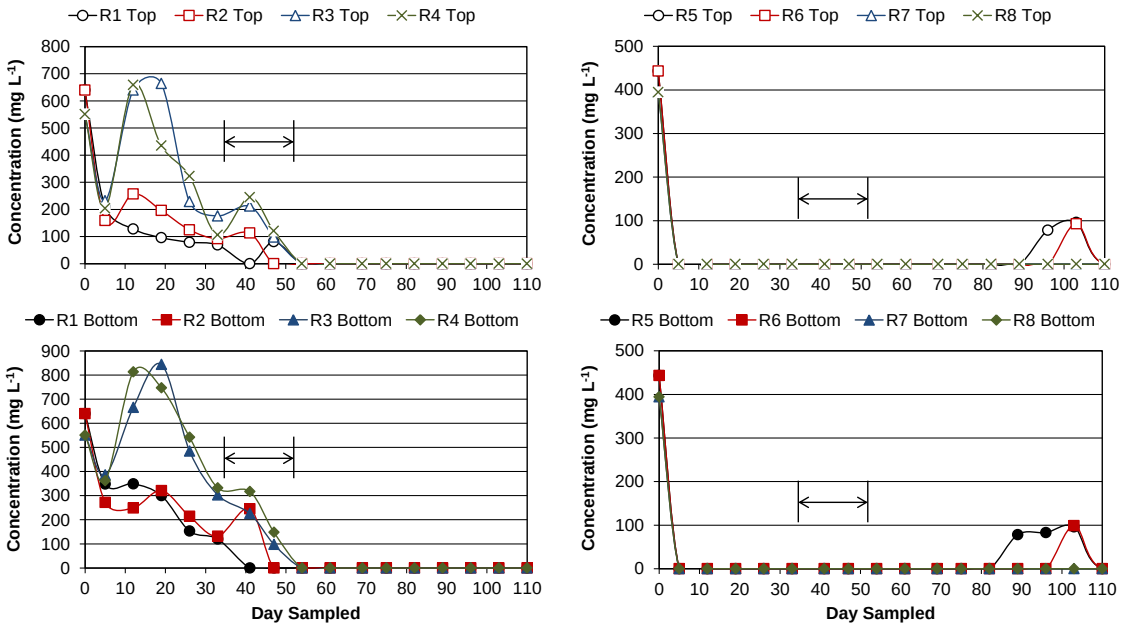

Figure 4.5. Comparison of butyric acid concentrations in the four sources. Top left: Top layer of untreated. Top Right: Top layer of treated. Bottom Left: Bottom layer of untreated. Bottom Right: Bottom layer of treated. Arrow indicates time of anaerobic conditions.

4.3.3.5 2-methylbutyric Acid

The dynamics of 2-methylbutyric acid were similar to that of butyric acid over the 3-month period. For reactors containing raw manure, there was again a decline in concentration within 5 days of storage. This was followed by a period of stable concentration (near 100 mg L^{-1}) until day 69 when the concentration declined to 0

mg L^{-1}. The maximum concentration was the initial 637 mg L^{-1} in the container. This was also the maximum concentration of 2-methylbutyric acid for all manures. The dynamics of 2-methylbutyric were very similar to butyric acid in the influent. Reactors containing AD influent experienced the same decline within 5 days followed by a rapid increase, peaking around day 19. A small peak again occurred during anaerobic conditions (day 41), but concentrations reached 0 mg L^{-1} by day 69. The maximum concentration was the initial container concentration, 473 mg L^{-1}. For both treated sources, 2-methylbutyric acid concentrations declined sharply after 5 days and stayed very low until reaching 0 mg L^{-1} around day 54 (the first sample taken once air was restored after anaerobic conditions). The maximum concentrations were the initial container concentrations of 426 mg L^{-1} and 384 mg L^{-1} for effluent and SS effluent, respectively. (Figure 4.6)

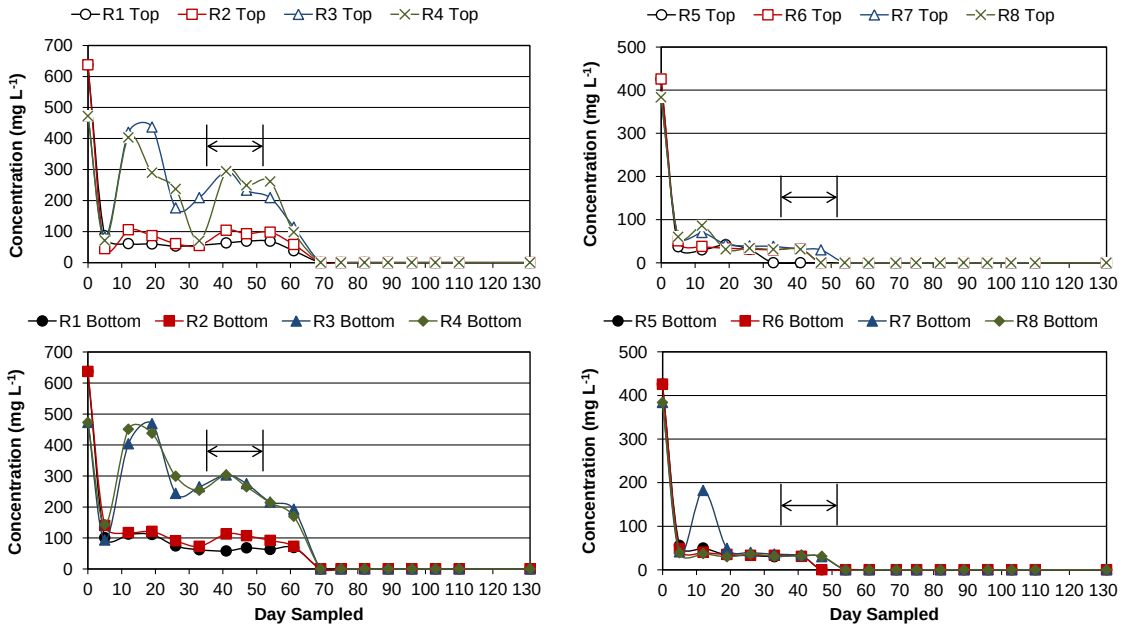

Figure 4.6. Comparison of 2-methylbutyric acid concentrations in the four sources. Top left: Top layer of untreated. Top Right: Top layer of treated. Bottom Left: Bottom layer of untreated. Bottom Right: Bottom layer of treated. Arrow indicates time of anaerobic conditions.

4.3.3.1 Isobutyric Acid

Analysis of VFA using gas chromatography allowed for the quantification of isobutyric acid in samples. The six sample sets that were run on both the GC and HPLC were from day 5, 12, 19, 26, 34 and 96 of storage. Looking at the results from the samples from the first five weeks of storage revealed that the highest concentrations of isobutyric acid were present in AD influent, averaging 180 mg L^{-1}. Concentrations in raw manure averaged 87 mg L^{-1}, while the average concentrations in treated manure were below 27 mg L^{-1} for the first 5 weeks. Isobutyric acid is produced from the breakdown of protein, which suggests that the substrates added to influent contained some protein. The concentrations of isobutyric acid were 0 mg L^{-1} in all sources and layers on day 96.

4.3.4 Effects of Manure Sources and Manure Treatment on VFA Concentrations

4.3.4.1 Effect of Pre-Consumer Wastes on VFA in AD Influent

The AD influent (R3 and R4) had the highest consistent VFA concentrations among all reactors (Table 4.5). Their total VFA of 1724±1832 mg L^{-1} (mean±standard deviation) during the entire study was almost twice as high as that from the raw manure (1043±1140 mg L^{-1}). This difference was not as significant compared with the first storage test, but the VFA concentrations were lower overall in this second study. This difference between the first and second storage tests demonstrated that the type and amount of pre-consumer wastes for dairy manure co-digestion had a significant effect on VFA fed into the digester.

4.3.4.2 Effect of AD and Separation of Solids on VFA in Stored Manure

The total VFA (sum of four VFA) presented in Table 4.5 and Figure 4.7 clearly show differences among various manure sources. In general, the manure before AD (R1 to R4) had significantly higher total VFA compared with the manure after AD ($P<0.05$). Additionally, concentrations of all individual VFA in the treated manure never exceeded 630 mg L^{-1} (Figure 4.3 to Figure 4.6) and were significantly lower than in untreated manure. This demonstrated that the Qualco

AD system greatly reduced VFA concentrations in stored manure. Although AD helped to reduce VFA concentrations, separation of solids did not seem to make a significant reduction in VFA when looking at the treated sources. The average total concentration for all 4 VFA was higher in SS effluent (256±409 mg L^{-1}) than AD effluent (239±442 mg L^{-1}). However, due to the limitation of quantifying only four VFA in this study, future investigations are needed to determine the effect of AD and separation of effluent solid on other VFA that exist in dairy manure.

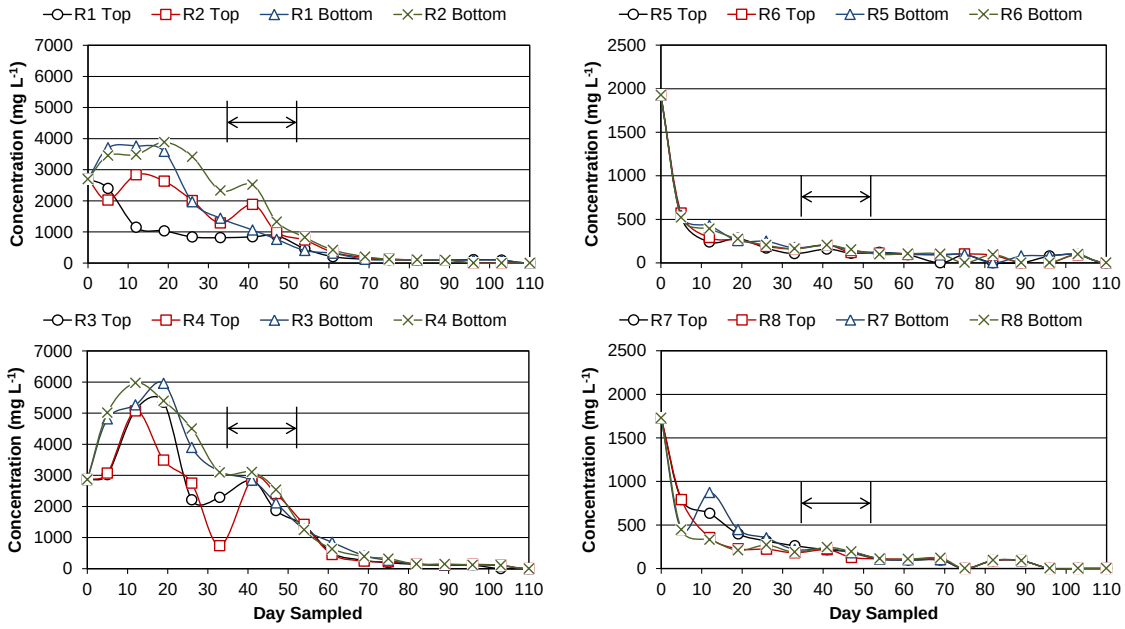

Figure 4.7. Variations of the total VFA concentrations in the top and bottom layers in eight reactors. Top Left: Raw manure. Top Right: AD effluent Bottom Left: AD Influent. Bottom Right: SS effluent. Arrow indicates time of anaerobic conditions.

4.3.5 Statistical Comparison of GC and HPLC

4.3.5.1 Comparison of Results

The results of the t-test comparison of the six sample sets that were run on both the GC and HPLC revealed significant differences in results for certain volatile fatty acids ($p<0.05$). For acetic acid, no significant differences were found

between concentrations from HPLC and GC analysis except for day 34 samples. For propionic acid concentrations, significant differences were found between samples on days 26, 34 and 96 of storage. The comparison of butyric acid concentrations revealed the largest difference between the two analysis methods. All sets were significantly different except for the sample from day 34. This occurred because the HPLC did not detect butyric acid in the samples from AD-treated manure while the GC detected very small amounts. Comparison of 2-methylbutyric acid concentrations showed significant differences for the samples from day 5, 26 and 34.

Table 4.6 displays the percent difference between concentrations for all six samples within the same source of manure for each VFA (when concentrations from either analysis method were not equal to 0 mg L^{-1}). The results revealed that the difference tended to be smaller for samples with higher concentrations (>100 mg L^{-1}) suggesting that the HPLC was unable to quantify low concentrations of VFA as the GC.

Table 4.6. Comparison of VFA concentration from GC and HPLC analysis.

Manure Source	% Difference			
	Acetic	Propionic	Butyric	2-Methylbutyric
Raw	24	23	21	16
Influent	140	28	14	20
Effluent	30	31	-	26
Effluent SS	74	27	-	34

4.3.5.2 Discussion of HPLC and GC methods

The differences in results from each method may have occurred due to a number of reasons. Several potential problems exist just at the sample vial level. Manure samples may not have been identical when splitting into separate vials. For good peak area reproducibility, samples must be homogeneous. Layering may have occurred because samples were frozen prior to analysis and may have been

poorly mixed (Barwick 1999).The GC had trouble quantifying VFA when multiple samples were run consecutively. If samples are not properly cleaned before running, the GC inlet liners can get easily clogged, affecting the results.

The differences may also be due to the analysis methods themselves. The GC is more sensitive and was able to quantify concentrations to lower amounts than the HPLC. For the HPLC, refractive index (RI) detection may be affected by "changes in solvent composition, pressure and temperature" (Barwick 1999). More studies are needed for better comparison of the two methods.

4.4 Conclusions

1. Acetic acid was dominant in all manure sources: raw manure, AD influent, AD effluent and SS effluent. Acetic acid accounted for between 66% and 80% of the total VFA monitored in all four sources. Acetic acid concentrations in treated manure and in the bottom layers of untreated manure demonstrated a general decrease in the study, while concentrations in the top layers of untreated manure demonstrated more irregular changes, which may have been due to the air compressor malfunction.
2. Propionic acid was the second most dominant VFA and accounted for 8% to 20 % of untreated manure, but <9% of treated manure.
3. Butyric acid accounted for less than 6% of the four VFA in raw, influent, and SS effluent manure but accounted for 10% to 22% in effluent manure.
4. Concentrations of 2-methylbutyric acid were the lowest among the 4 VFA quantified in this study in untreated manure. In treated manure, 2-methylbutyric acid concentrations were similar to that of propionic acid but greater than butyric acid concentrations.
5. Formic acid was not present in any of the samples in this study due to the different pre-consumer wastes in the influent from the first storage test.
6. The pre-consumer wastes mixed with dairy manure increased the total VFA by almost 150% compared with raw manure.

7. Concentrations of the total VFA in treated manure exhibited a general decreasing trend over the three months of storage. However, concentrations in untreated manure were sporadic and less predictable.
8. Because VFA concentrations were significantly lower in the group of treated manure than in the group of untreated manure, this study confirmed that AD significantly reduced VFA from dairy manure and pre-consumer wastes.
9. Most of the four VFA in the four different manure sources exhibited highly variable temporal and spatial differences. The characteristics of VFA revealed in this study were more complex than previously reported. This complexity makes it difficult to reliably model and predict the concentrations and compositions of VFA in dairy manure.
10. Solid-liquid separation did not have a significant impact on VFA production during storage in this lab-scale study.
11. Analysis using HPLC and GC methods yielded significantly different VFA concentrations from the same sample, demonstrating a necessity of research on methodologies of VFA analysis.

CHAPTER 5. GENERAL DISCUSSION

5.1 Effects of AD and Post-AD Solids-Liquid Separation on VFA

Both dairy manure storage tests demonstrated that AD significantly reduced VFA from dairy manure and pre-consumer wastes. Concentrations of VFA were reduced by 98% and 86% in the first and second storage tests, respectively. To assess the impact that AD had on VFA compared to odor, a threshold of unequivocal unacceptability of odor at 520 mg L^{-1} (total VFA) will be used (Ndegwa 2003). In the first test, although VFA were reduced by AD-treatment, concentrations in the effluent manure persisted above this threshold in the bottom layer of manure for around 35 days, and again reached concentrations above the threshold in the last two weeks of storage. In the second test, VFA concentrations in the effluent manure reached an acceptable level within 5 to 20 days of storage. In both tests, concentrations of VFA in raw manure remained above the threshold for more than 60 days of storage. The VFA concentrations in the influent manure in the first test remained above the odor threshold for the entire 3-month storage period, while concentrations in influent manure in the second test reached an acceptable level by day 60. The results from both tests revealed that even after AD-treatment, the manure had the potential to cause odor problems in the first 20-30 days of storage, but ultimately the time to reach an acceptable level was reduced by AD (Figure 5.1 and Figure 5.2).

Solids-liquid separation provided a further reduction of VFA in the first test, but did not seem to have a significant effect in the second test. Again looking at VFA concentrations compared to the odor threshold, solid-liquid separation seemed to reduce the time that concentrations remained above the odor threshold in the

first test. In contrast, concentrations of VFA in SS effluent manure remained above the threshold longer than effluent manure during the second storage test. Past studies have shown that there may be a need to remove very fine particles to achieve a significant odor reduction (Ndegwa 2003).

5.2 Dynamics of the Changes of VFA in Untreated Manure

The VFA concentrations in untreated manure sources were significantly smaller in the second storage test compared with the first test (Table 5.1). Differences in raw manure do not seem to be from differences in %TS because raw manure in the second test had a larger %TS which would suggest a greater potential to produce VFA. The difference may be due to seasonal variability in VFA production with lower concentrations in manure collected during winter months (Merrill and Halverson 2002). Although concentrations differed, raw manure in both tests was dominated by acetic acid followed by propionic acid. Similar temporal changes in VFA occurred in raw manure in both tests with acetic acid maintaining a relatively high concentration for the first 40 days of storage followed by a steady decline in concentration. The rate at which the individual VFA degraded seemed to be proportional to their original concentration in raw manure.

The significant difference in VFA concentrations between the AD influent from each study may be explained by the already low VFA concentrations in raw manure for the second test as well as the difference in co-substrates. Based on the records kept at the digester, the types of substrates added were almost identical although the amount of each differed (Table 5.2).

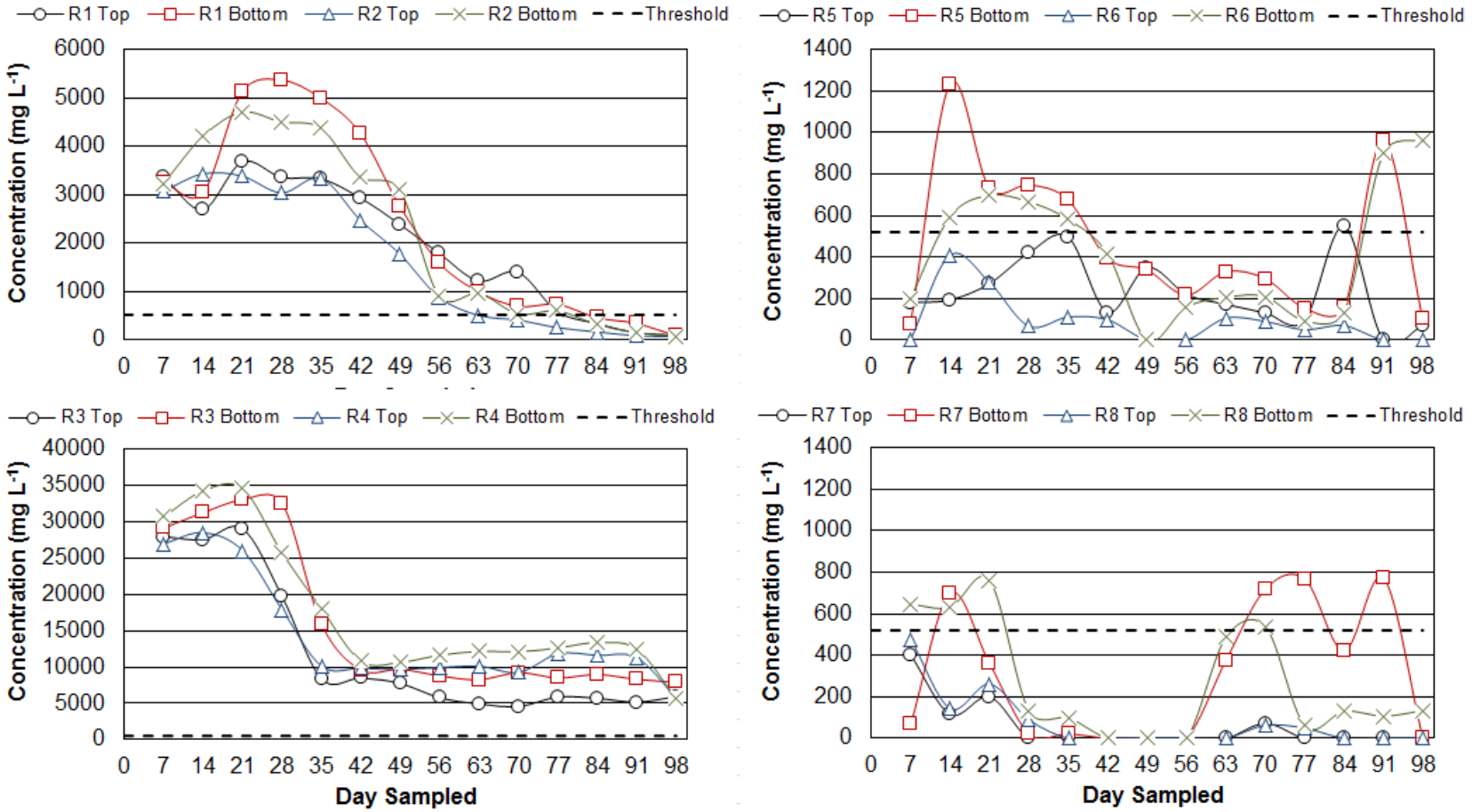

Figure 5.1. Total volatile fatty acid concentrations during storage in the first test. Top Left: Raw manure. Top Right: AD effluent. Bottom Left: AD Influent. Bottom Right: SS effluent.

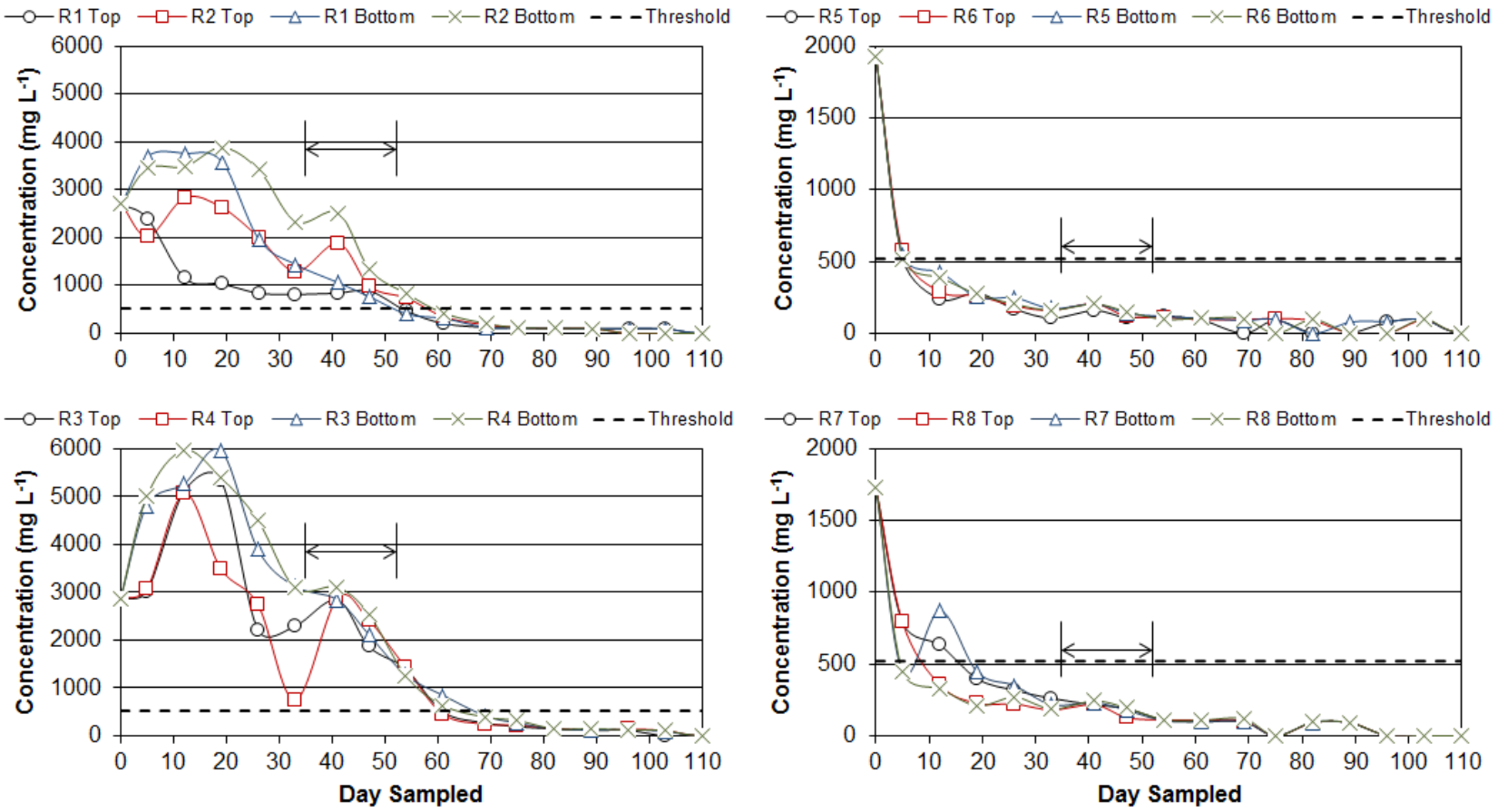

Figure 5.2. Total volatile fatty acid concentrations during storage in the second test. Top Left: Raw manure. Top Right: AD effluent. Bottom Left: AD influent. Bottom Right: SS effluent

Table 5.1. Summary of VFA concentrations (mean ± standard variation) in untreated manure for both storage tests.

Reactor	Layer	Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid		2-Methylbutyric	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1	T	20±75	0±0	1298±967	422±410	424±232	103±190	130±142	72±154	82±48	66±146
1	B	107±180	0±0	1424±1346	714±845	661±347	234±355	129±145	106±184	88±60	75±147
2	T	101±122	0±0	1098±941	639±664	285±307	195±244	79±85	88±160	74±49	75±147
2	B	135±245	0±0	1318±1228	853±951	552±337	328±368	122±127	115±177	90±67	87±147
1 and 2	T and B	91±169	0±0	1284±1109	657±694	480±332	215±272	115±125	95±164	84±55	76±146
3	T	5545±9248	0±0	2300±534	940±939	1488±354	328±477	2284±1104	156±231	294±201	147±168
3	B	7516±11197	0±0	2992±481	1082±1131	1813±178	455±527	3047±1110	198±276	424±274	163±175
4	T	5117±8775	0±0	2969±933	865±866	2103±244	303±413	3727±1631	147±213	280±165	136±159
4	B	7386±11226	0±0	3399±1010	1150±1173	2265±422	450±552	4106±1756	212±284	317±194	167±176
3 and 4	T and B	6391±9950	0±0	2915±853	1009±1006	1917±427	384±482	3291±1558	178±247	329±214	153±167

The real difference can be seen from the high %TS of influent manure in the first test (6% compared to 3% in the second test) and the presence of formic acid in the first test but not the second (Table 5.1). The retention time of the digester is a theoretical number of days, and with a modified plug-flow digester, fibrous material may tend to stay back allowing more liquid that's been well digested to flow. At the time of sampling influent manure for the first test, there may have been a high amount of fibrous feedstock like corn silage present, which would explain the high %TS and the production of formic acid. In addition, a much larger amount of "Trap" (waste that's high in moisture) was present in the influent manure from the second test, which would help to dilute the dairy manure. Based on the theoretical amounts of substrate present in the manure, a higher ratio of dairy manure to pre-consumer waste produced higher concentrations of VFA. The differences in substrates also affected the dynamics, quantities and formation between individual VFA. Influent manure in the first test had extremely high amounts of formic acid, followed by butyric and acetic acids, while in the second test showed similar dynamics as raw manure, dominated by only acetic acid followed by propionic acid. The substrates added in the second test seemed to only amplify the individual VFA already present in raw manure, without affecting the dynamics of the changes.

Table 5.2. Overview of "pre-consumer" wastes and dairy manure input into the anaerobic digester in both tests, %.

	Blood	Fish	Trap	Biodiesel	Bedding	Dairy Manure
Influent [(1)]						
Test 1	5.9	0	4	0	0	90.1
Test 2	6.4	0	21.7	0	4.5	64.8
Effluent [(2)]						
Test 1	6.9	1.2	23.6	0	0	68.3
Test 2	2.7	0	21	2.7	8.8	67.4

[(1)] Substrate concentrations based on inputs on day of collection. [(2)] Substrate concentrations based on theoretical retention time of 16 days and recorded inputs.

5.3 Comparison of VFA in Treated Manure

Concentrations of VFA in treated manure were higher in the second storage test compared with the first test (Table 5.3). This may suggest that more undigested material was present in manure from the second test. All treated manure in both tests was dominated by acetic acid, with some formic acid in the first storage test. Changes in VFA during storage of treated manure were significantly different between the storage tests. The first test showed irregular, almost sporadic, changes in concentrations of acetic acid during storage. In the second test, concentrations of all VFA declined once manure was stored in the reactors, and demonstrated a steady decline.

Table 5.3. Summary of VFA concentrations (mean ± standard variation) in treated manure for both storage tests.

Reactor	Layer	Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid		2-Methylbutyric	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
5	T	108±166	0±0	89±78	122±162	18±64	34±101	6±10	34±106	13±20	31±100
5	B	90±242	0±0	282±196	143±166	42±58	36±101	14±17	39±106	30±26	37±99
6	T	2±4	0±0	76±96	140±161	1±4	34±101	4±9	30±105	8±14	36±99
6	B	124±312	0±0	237±186	141±163	16±23	34±101	13±16	30±106	26±25	36±99
5 and 6	T and B	90±214	0±0	171±171	137±161	19±46	34±101	9±14	33±105	19±23	35±99
7	T	2±7	0±0	42±92	180±199	1±4	38±86	4±9	22±93	6±13	39±89
7	B	99±251	0±0	175±231	173±189	6±13	36±85	7±15	22±93	15±17	44±95
8	T	0±0	0±0	61±111	146±180	2±8	37±85	6±10	22±93	8±13	36±90
8	B	0±0	0±0	225±238	138±147	12±23	35±83	14±18	22±93	16±21	34±89
7 and 8	T and B	25±130	0±0	126±192	159±171	5±14	36±84	8±14	22±93	11±16	38±90

CHAPTER 6. GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 General Conclusions

1. An anaerobic digester on a dairy farm utilizing co-digestion with different substrates displayed very different composition in influent manure, but these changes did not seem to affect digester efficiency. The VFA concentrations were significantly lower in the treated manure than in the group of untreated manure in both studies, demonstrating that AD significantly reduced VFA from dairy manure and pre-consumer wastes.
2. The dominant VFA in raw dairy manure was acetic acid followed by propionic acid in both tests. Acetic acid was also the dominant VFA present in treated manure in both tests. This characteristic of acetic acid in dairy manure confirmed the past research results reported in the literature.
3. The dominant VFA in AD influent depended on the substrates added to raw manure. Formic and butyric acids were the predominant VFA present in influent in the first test followed by acetic acid. Acetic acid was the predominant VFA present in influent in the second test followed by propionic acid.
4. The total concentration of VFA in treated manure reached concentrations above the threshold of unequivocal unacceptability of odor for VFA during the first 20-30 days of storage, but AD helped reduce the time for concentrations to reach an acceptable level. Therefore, AD of dairy manure and pre-consumer wastes may have the potential of reducing odor emissions.
5. Concentrations of the total VFA in treated manure exhibited a general decreasing trend over the three months of storage. However, concentrations in untreated manure were sporadic and less predictable.

6. The VFA monitored exhibited highly variable temporal and spatial variations in both studies. The complexity of characteristics of VFA just within this study displays the difficulty in predicting concentrations and compositions of VFA in dairy manure when co-digestion is utilized.

6.2 Recommendations for Future Research

- Basic equipment maintenance before initiating the experiment may help prevent problems such as the breaking of the air compressor.
- If GC analysis is desired for manure samples in the future, a better method should be used for developing standards and preparing the samples before GC use.
- Manually adjusting the height of the pH probe in the top layer of manure should be avoided. Perhaps a method to float the probe on the manure surface could be developed.
- When manure is being loaded in the reactors, initially, more attention should be put into mixing the manure to prevent differences in solid content as seen by the second test in effluent and effluent SS manure. The containers may need to be mixed for a longer time before attempting to fill reactors.
- The storage tests provided comprehensive monitoring data of 3 different pollutant types. The VFA data from both storage tests may be further analyzed by taking into account the other two components: odor and gases, This will allow more scientific information to be reviewed as well as improve the evaluation of the efficiency of waste treatment.
- It will be beneficial to more accurately monitor and record the actual input of manure and pre-consumer substrates to the digester complex for scientific research purposes. This study revealed some discrepancies between the theoretical substrates present in manure and what was actually seen in samples.

- The method of using VFA as an odor indicator may be validated by conducting more studies on concentrations of individual VFA and odor during dairy manure storage.
- Future studies should focus on the mechanisms of VFA changes under variable manure storage conditions as well as the effects of co-digestion materials related to post-AD storage.

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